

Review Article

Molecular pathogenesis of progression and recurrence in breast phyllodes tumors

Ana Richelia Jara-Lazaro, Puay Hoon Tan

Department of Pathology, Singapore General Hospital, Outram Road, Singapore 169608

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Abstract: Breast phyllodes tumors are rare fibroepithelial neoplasms that need to be distinguished from the common morphologically similar fibroadenomas, because phyllodes tumors can recur and progress to malignancy. Their potentially recurring and metastasizing behavior is attributed to their stromal characteristics, for which categorization between benign, borderline and malignant tumors have not been universally established. Previous clonality studies revealing monoclonal stromal cells versus a polyclonal epithelial component theorized that phyllodes tumors are mainly stromal neoplasms, possibly arising from fibroadenomas. More recent chromosomal imbalances in both epithelium and stroma have challenged this theory to favor neoplasia of both epithelium and stroma, with initial interdependence between the two components. Inverse correlations between epithelial and stromal overexpression for various biological markers like estrogen receptor, p53, c-kit, Ki-67, endothelin-1, epidermal growth factor receptor, heparan sulfate, in addition to findings of epithelial Wnt signalling with stromal insulin growth factors and beta-catenin expression, suggest an initial epithelial-stromal interdependence at the benign phase. Upon progression to malignancy, the stroma is hypothesized to assume an autonomous growth overriding any epithelial influence. Frequent genetic alterations are chromosomal gains of 1q and losses at chromosome 13. Acquisition of new genetic imbalances within the tumor consistent with intratumoral heterogeneity, and subclones within histologically benign phyllodes tumors that recur or metastasize are the current theories explaining these tumors' unpredictable clinical behavior.

Key words: Molecular pathogenesis; phyllodes tumors; epithelial-stromal interactions; biological markers; genetic alterations; subclones

Introduction

Phyllodes tumors have been considered rare fibroepithelial neoplasms that comprise 0.3 to 1.5% of breast tumors in western countries [1]. In Singapore, when compared to breast cancers, its incidence stands at 6.92% [2], suggesting its higher frequency among Asian women.

Despite the biphasic histomorphologic pattern phyllodes tumors share with the more common benign fibroadenoma, its notoriety is in its propensity to recur, and also possibly metastasize. This tendency towards a locally aggressive behavior has been related to its distinct histologic features, such as an increased albeit heterogeneous stromal cellularity rendering its leaf-like architectural

pattern, the variable amount of stromal cell atypia and increased mitotic figures, possible malignant metaplastic changes within the stroma, and pushing or infiltrative borders [1,2]. Grading and prognostication have been dependent on the presence and severity of these stromal features. Whether to classify these as low grade or high grade tumors, or as benign, borderline, malignant phyllodes tumors (**Figures 1, 2, 3**) is not universally established, nor have the histologic cut-offs for its tiers also been uniformly defined [1,2,3,4].

The World Health Organization has recommended the term 'phyllodes tumour', as derived from its original name 'cystosarcoma phyllodes' – termed from its leaf-like fleshy gross appearance [1]. Although a sarcoma-like stroma is seen in malignant phyllodes, the

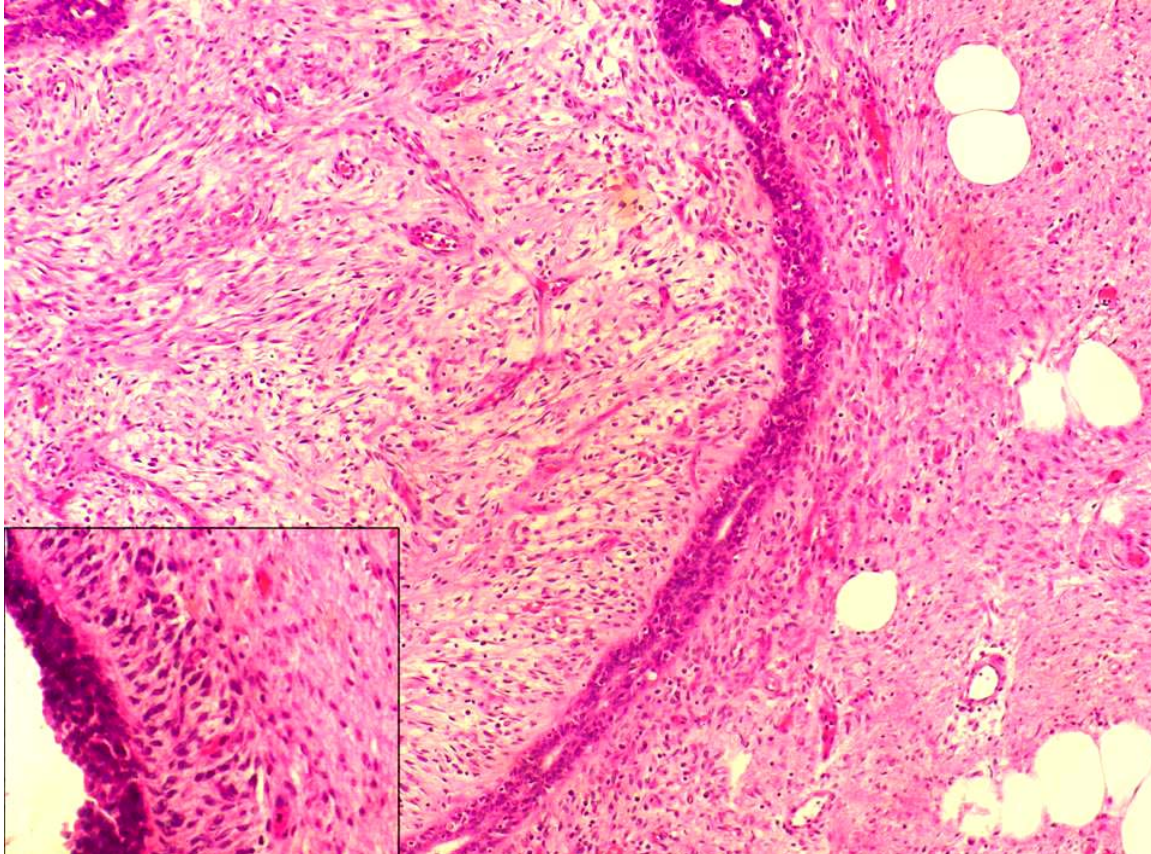


Figure 1: Benign phyllodes tumor with an elongated meandering stretch of epithelium which forms the semblance of a frond. Inset shows perithelial accentuated stromal cellularity.

great majority of phyllodes do not harbor this histology, and they metastasize hematogenously like sarcomas in only a minority of cases [1]. It is therefore preferred that the term phyllodes tumor is used instead of cystosarcoma phyllodes.

Advances in immunohistochemical and molecular methods have shed light on the biological nature of this neoplasm. While still fraught with many questions and occasionally conflicting results, these studies pave the way for further understanding the pathogenesis and potentially malignant behavior of this prognostically unpredictable neoplasm.

The phyllodes tumor and the fibroadenoma

Because phyllodes tumors tend to grow more rapidly and more sizeably than fibroadenomas, yet can harbor the same intracanalicular structures as the latter, they were at one time, considered synonymous with giant

fibroadenomas by some. The more commonly benign phyllodes tumor's mild stromal hypercellularity can histologically overlap with the cellular fibroadenoma [1, 3]. Frequently, they are morphologically indistinguishable on limited tissues like needle core biopsies [1, 3]. In the study by Tan et al, fibroadenomas occurred synchronously in 4.2% of 335 phyllodes tumors. Thus, the phyllodes tumor and the fibroadenoma have become frequently compared entities when their molecular profiles are studied, as authors aimed to link the two entities, or explain how they can behave so differently despite their morphologic similarities [5, 6].

Noguchi et al's first study on clonal analysis of the fibroadenoma and phyllodes tumor, using gene amplification by polymerase chain reaction, showed that fibroadenomas analyzed in the study were polyclonal in both epithelium and stroma, whereas the phyllodes tumor was polyclonal in epithelial cells and monoclonal in

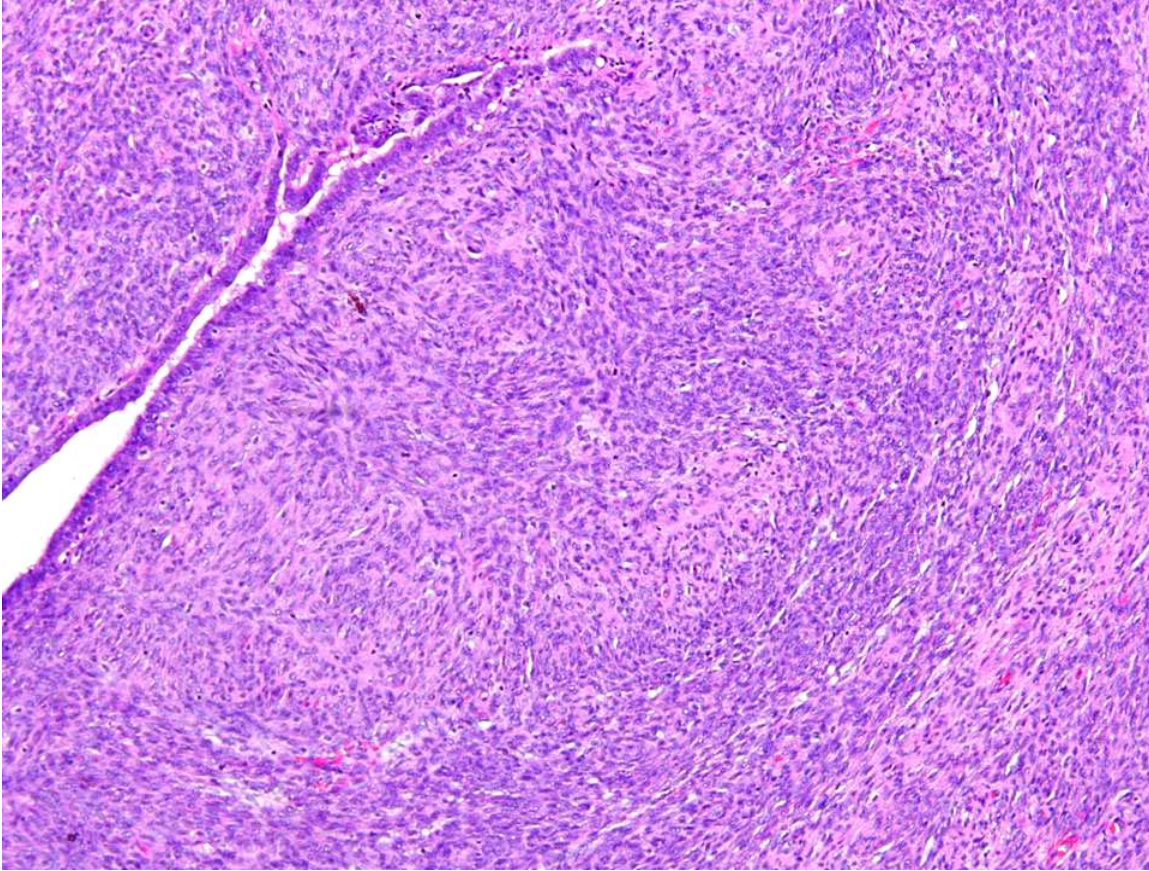


Figure 2: Borderline phyllodes tumor with increased stromal cellularity composed of fairly uniform cells.

stromal cells [5]. Their hypothesis then was that the histogenesis of these two tumors is related, and that the neoplastic component in phyllodes is the stroma [5]. They further speculated that in the unlikelihood of a de novo monoclonal growth of stromal cells, the phyllodes would begin as a fibroadenoma (which they concluded was hyperplasia of a lobule), with somatic mutation in its stromal cells to form a phyllodes [5]. In another study, the same authors reinforced this latter hypothesis through another clonal analysis study, which revealed that the three primary fibroadenomas that ‘progressed’ to become phyllodes tumors were all monoclonal in origin, with inactivation of the same alleles of their androgen receptors [6].

At this point, these studies promoted the general belief that the phyllodes tumor was mainly a stromal neoplasm, with the epithelial component behaving as an innocent bystander as the stroma undergoes proliferation. In

addition, they theorized that the fibroadenoma was a possible precursor or progenitor lesion of the phyllodes tumor [6], an apparent possibility which, to date, cannot be completely ruled out in some cases.

Challenging the epithelium’s “innocence”

This stroma-driven belief has been refuted by several later studies [7,8,9,10,11,12,13]. Since many phyllodes tumors also do harbor epithelial hyperplasias, lobular neoplasias and infiltrating ductal carcinomas [7], a similarly proliferating epithelial component within phyllodes tumors raised the question on how “innocent” its epithelial component really is. One study cited the comparative genomic hybridization (CGH) analysis of 18 fresh-frozen phyllodes tumors which revealed gain of 1q and loss of material on 3p as the two most common chromosomal abnormalities, similar to earlier reports of such abnormalities in breast carcinoma [7]. As this common genetic

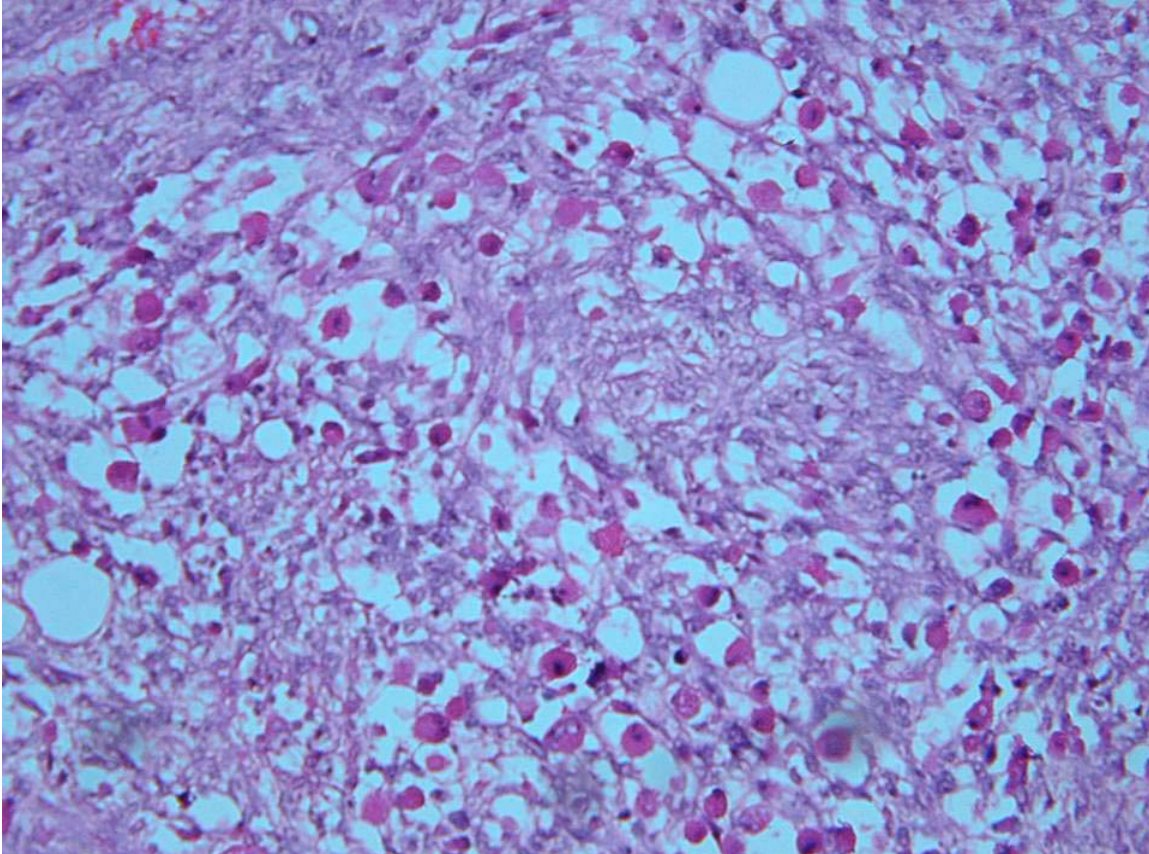


Figure 3: Rhabdomyosarcomatous heterologous elements in the stroma of a malignant phyllodes tumor.

profile suggested similar pathogenesis for both phyllodes tumor and breast carcinoma, Sawyer et al proceeded to perform allelic imbalance (AI) assessments in 47 phyllodes tumors and 78 breast carcinomas, using microsatellites on chromosomes 1q and 3p, comparing their areas of gains and losses [7]. In the phyllodes tumors, 14 of 46 (30%) showed AI at one or more markers on 1q and 10 of 42 (24%) showed AI on 3p; and the breast carcinomas showed higher rates of AI of 67% in 1q and 40% on 3p [7]. The AI was detected in the stroma only of 8 phyllodes tumors, 4 of which were at markers on 1q (n=1), and in 3p (n=3); 8 other phyllodes tumors showed epithelium-specific AI on 1q (n=4), and imbalances on 3p (n=3), and AI on both arms (n=1); and in 5 phyllodes tumors, both epithelium and stroma showed imbalances of the same allele at the same marker in 4 cases [7]. With the highest frequency of AI toward the 1q telomere in the stroma and epithelium of the phyllodes tumors

and in the breast cancers in the study, the findings suggested that in some phyllodes tumors, both stroma and epithelium are neoplastic [7]. Various immunohistochemical and genetic studies would later pursue this hypothesis.

The few discordant findings between these two components in individual phyllodes tumors in the study, (i.e. maximum imbalance at D3S1300 in the stroma, and at D3S1293 in the epithelium along the 3p chromosome), led to further speculation regarding clonality – either both tumour components have independent clonal origins, or they originate from the same clone but acquire different mutations during tumour progression [7]. The study deemed the latter more theoretically plausible.

Identifying genetic changes of phyllodes tumors

Molecular pathogenesis of breast phyllodes tumors

Other studies concurred with the finding of gain of 1q, but did not reveal allelic loss at the 3p loci [8]. 3p14 is the location of the FHIT (fragile histidine triad) gene, a tumor suppressor gene which is said to be abnormally transcribed in several primary carcinomas of the lung, gastro-intestinal tract, lung and also breast [8]. The DNA mismatch repair gene homologue hMLH1 also resides in this location, and is the mutated gene in hereditary nonpolyposis colon cancer, and bladder cancer [8]. In the absence of consistent losses at any of the 3p loci, this abnormality might not be as significant in phyllodes tumors, as in primary cancers from other sites.

A more recent study investigated further the genetic imbalances characterizing phyllodes tumors, mainly to determine how these might help evaluate their malignant potential [9]. Results revealed that the most frequent gains involved again chromosome arm 1q (12 of 30 cases), as well as chromosomes 5 (9 of 30) and 18 (5 of 30) [9]. Chromosomal losses were also most frequently found at 13q (7 of 30), 6q (9 of 30), 10p (8 of 30), and 12q (6 of 30) [9]. Chromosome 13q14.2 harbors the RB1 gene, a tumor suppressor gene which is suspected to be the target of the deletions. Chromosomal 1q gain and/or 13q loss were the most statistically significant findings in the tumors, and were said to be hallmark alterations in phyllodes tumors. These recurrent chromosome imbalances were identified in 83% (25 of 30) of the tumors, which involved 55% of morphologically benign, compared to 91% of borderline and 100% of malignant cases [9]. The rest of the benign phyllodes tumors did not demonstrate any chromosomal changes [9]. Based on the number of chromosome imbalances, their findings suggested that benign tumors could be separated from borderline and malignant ones [9]. Benign tumors showed a median of one chromosomal change (range 0-3), whereas the borderline and malignant tumors showed median number of 6 chromosomal changes (range: 0-13, and 1-20, respectively) [9]. No statistical difference between borderline and malignant tumors was seen [9]. Another observation in this study was that tumors with no or few chromosome imbalances were those characterized by nuclear size of <50 μ m³ and/or a mitotic rate of <3/10 high power fields (hpf), and/or a

cellularity <100 nuclei/1hpf, features which they evaluated using cell digital image analysis [9]. Furthermore, FISH genomic amplification was also observed as MDM2 and MYC were amplified in one phyllodes tumor each [9]. MYC is amplified in a number of epithelial tumors, including breast carcinoma [9]. MDM2 is a negative regulator of p53, and its amplification in combination with frequent loss of 13q suggested a link between phyllodes tumors and sarcomas [9].

While genetic expression is evidently involved in tumorigenesis, its exposure to an interplay of extracellular factors would comprise the development and progression of breast cancers as a whole. Particularly in phyllodes tumors, the interplay would be believed now to be between its two main components: the epithelium and the stroma.

Epithelial-stromal interactions

Histomorphology alone hints at epithelial participation in the stromal expansion of phyllodes, with frequent perithelial stromal accentuation (**Figure 1**) described as one of its suggestive features [3]. The presence of mitotic figures within the periductal stroma as opposed to stroma away from the epithelium in benign phyllodes tumors, also strongly suggests that stromal growth may actually be dependent on the epithelium [10]. In an experiment using a morphometric technique, this increased mitotic activity in the periductal stroma was attributed to the epithelium which was presumed to produce a humoral factor with a maximum range of action of approximately 200 μ m [10]. Since breast epithelium is said to promote estrogen-dependent stimulation of fibroblast DNA synthesis in normal breast development, as a result of growth factor-mediated tissue interaction between the developing epithelium and surrounding stroma [10], this interaction presumably transpires variably in neoplastic growth as well.

One of the first studies to explore this epithelial-stromal interaction in phyllodes tumors examined the Wnt-APC-beta-catenin pathway using beta-catenin and cyclin D1 immunohistochemistry on 119 phyllodes tumors [11]. The Wnt pathway is a cell signal transduction pathway that results in beta-catenin stabilization and its translocation to

Molecular pathogenesis of breast phyllodes tumors

the nucleus to activate specific genes [11]. 86 tumors (72%) showed stromal nuclear staining by beta-catenin, with a periductal accentuation noted, suggesting the epithelium-dependent proliferative capacity of the stromal cells in benign phyllodes tumors [11]. On the other hand, 7 of 8 malignant tumors in the study showed absent or weak stromal staining [11]. The strong beta-catenin stromal nuclear staining was associated with widespread epithelial Wnt5a mRNA overexpression assessed by in situ hybridization [11]. The findings suggested that stromal proliferation in benign phyllodes tumors is influenced by abnormalities in Wnt5a expression in the epithelium [11]. Furthermore, as there was no overexpression of beta-catenin in the epithelial cells in normal breast tissue and all phyllodes tumors, it was assumed that Wnt5a acts on stromal cells only. Upon progression to higher grade phyllodes, this stromal proliferation was explained to become independent from the Wnt pathway whose action is presumably derived from the epithelial component [11].

Because not all phyllodes tumors expressed epithelial Wnt overexpression in that series, an alternative explanation for the abnormal beta-catenin stromal accumulation was sought in the consequent study by the same authors, assessing Insulin-like Growth Factor (IGF)-I and IGF-II on fibroadenomas and phyllodes tumors [12]. IGF-I can supposedly activate the beta-catenin pathway, and IGF-II can also cause beta-catenin translocation to the nucleus [12]. Using in situ hybridization, 19 of 23 phyllodes tumors (83%) showed widespread stromal overexpression of IGF-II and 5 of 23 (22%) showed stromal expression by IGF-I [12]. The latter correlated with stromal nuclear beta-catenin staining, and had an association with Wnt5a expression [12]. This finding suggested that the effect of IGF-I and Wnt signaling may be additive or complementary, and not an alternative means of increasing nuclear beta-catenin expression in phyllodes tumors [12]. Since IGF expression occurred within the densely cellular stroma distant from the epithelium, it was also suggested that IGFs may be responsible for beta-catenin expression within expansile stroma, whereas epithelial WNT5a would be responsible mainly for sub-epithelial beta-catenin stromal expression [12]. Both IGF-I and beta-catenin were absent or reduced in malignant phyllodes, thus reinforcing the theory that the

pathway is not required upon autonomous stromal growth in malignant tumors. On the other hand, stromal expression of beta-catenin in all the fibroadenomas and in the majority of benign phyllodes tumors linked the two entities at the molecular level, making them similar lesions within the same biological continuum [12].

Further studies supported the epithelium-stroma interdependence. In a study using estrogen receptor (ER) immunohistochemistry, there was inverse correlation between epithelial ER expression and the stromal mitotic count, as the former also diminishes in the borderline and malignant phyllodes, compared with the benign [13]. This epithelial ER expression affirms its possible paracrine role on stromal proliferation in phyllodes tumors. Deriving from the theory of estrogenic influence on stroma in normal breast histogenesis, it may even be hypothesized that the epithelium may be the active initiator in the formation of the phyllodes tumor.

Of similar mediator role between epithelial and stromal components is endothelin-1 (ET-1), a vasoactive peptide, which is also said to have diverse paracrine and autocrine actions that induce mitosis in human breast fibroblasts, as well as carcinoma cells [14]. In one study, immunohistostaining for ET-1 was predominantly confined to the epithelium of all benign phyllodes tumors, 50% of borderline phyllodes, and 17% of malignant ones [14]. Diffuse myoepithelial nuclear staining was also seen [14]. Only focal positivity was seen in stromal cells of 2 of 16 benign tumors in that study, and diffuse stromal positivity was noted in 3 of 6 malignant ones [14]. Epithelial ET-1 also negatively correlated with mitotic count and stromal cellularity [14]. The decrease in epithelial ET-1 expression in malignant tumors combined with stromal positivity in some, was explained as either a possible up-regulation of stromal ET-1 receptors, or an increase in vascular endothelial growth factor expression and subsequent neovascularization [14], thus promoting tumor progression.

Malignant progression of phyllodes tumors

As the phyllodes tumour progresses to malignancy and a potentially recurring behavior, other molecular mechanisms involved may be deduced from various

Molecular pathogenesis of breast phyllodes tumors

biological markers used in studies comparing the benign, borderline and malignant tumors. One of the most widely studied is p53, a product of a tumor suppressor gene, located on chromosome 17p13.1 [15]. Its mutations are one of the most common genetic abnormalities in cancer, for which it is deemed a useful prognostic predictor, and its mutant forms are reflected by increased p53 staining on immunohistochemistry [15]. Stromal p53 expression has almost consistently been reported to increase significantly with

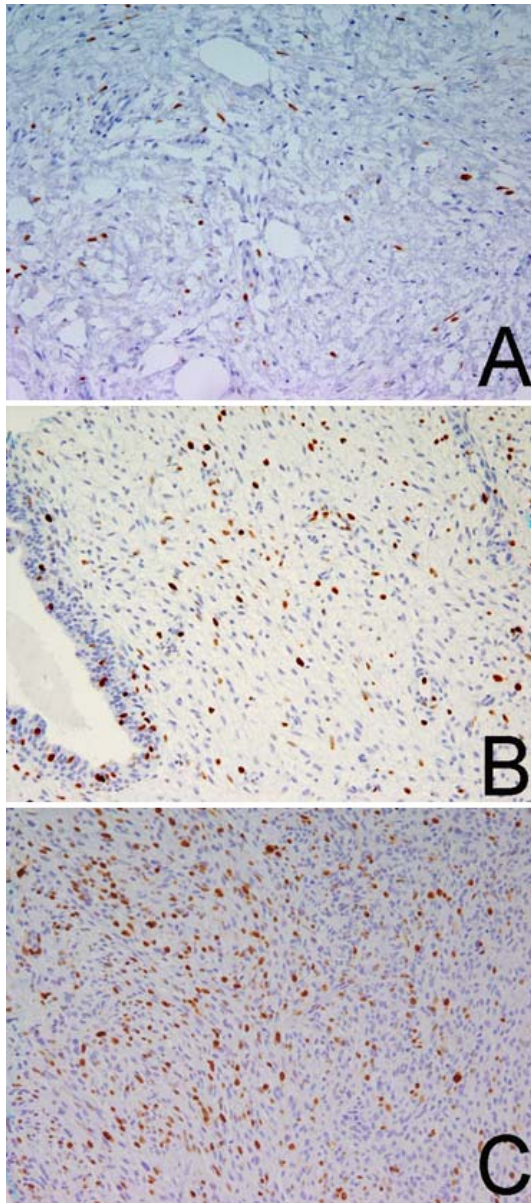


Figure 4: Increasing Mib1 immunohistochemical staining in (A), benign, (B) borderline, and (C) malignant phyllodes tumors.

phyllodes tumour grade [16], which is represented mainly by stromal hypercellularity and overgrowth in one study [15]. Moderate to strong p53 positivity was located at sites of peri-epithelial stromal condensation and atypia in 5 of 6 malignant phyllodes in another study, where all 20 fibroadenomas, all 9 benign phyllodes, and 1 malignant phyllodes tumour showed either negative to focally weak nuclear stromal positivity [17]. Although most studies report no correlation with recurrent disease [15, 16], one study found p53 expression an independent prognostic factor for disease-free survival in a multivariate analysis [18]. The significant increase in p53 expression would mostly be between benign phyllodes tumors and borderline to malignant tumors, with no significant difference in the latter two categories [15, 17]. Interestingly, one study also noted p53 immunostaining in a proportion of luminal epithelial and myoepithelial cells in its series of tissue microarrays [15]. The correlation between p53 epithelial and stromal staining again reminds of the epithelial-stromal interaction in phyllodes tumors, although the role of myoepithelial positivity is unclear.

Proliferation markers are considered useful predictors of tumor progression and cancer prognosis; and in phyllodes tumors, these have shown related findings with p53. MIB-1 is a monoclonal antibody, which reacts with Ki-67, a nuclear antigen expressed in the active phases of the cell cycle, with peak value during the G2M phase [16]. Ki-67 labeling indices have ranged from 1.3% to 4.7%, 6% to 26%, and 12% to 50%, for benign, borderline and malignant phyllodes tumors, respectively [14]. Paralleling similar correlations with increasing grade, particularly with stromal cellularity [16], like p53, Ki-67 also does not appear to be a reliable predictor of tumor recurrence, as labeling indices of recurrent tumors ranged from <1% to 60% in one study [14]. **Figure 4** shows increasing Ki-67 (MIB-1) immunohistochemical expression in benign, borderline and malignant phyllodes tumors. Other means to assess cellular proliferation, such as S phase fraction, measured by flow cytometry, would also show progressive increase from fibroadenoma, to benign, borderline and malignant phyllodes tumors, and was reported as an independent prognostic indicator [16].

Molecular pathogenesis of breast phyllodes tumors

Another marker is epidermal growth factor receptor (EGFR) [19], which is said to mediate tumor formation and progression pathways intracellularly, via ras-activated mitogen activated protein kinase, phosphatidylinositol 3-kinase/AKT and phospholipase C pathways that modulate cell motility, adhesion and proliferation [20]. Overexpressed in many other human malignancies, EGFR was likewise expressed in the stroma and myoepithelial cells of phyllodes tumors in one study, also significantly increasing with increasing tumor grade [19]. EGFR was further associated with stromal cellularity and overgrowth, nuclear pleomorphism, mitosis, infiltrative margins, and size [19]. The corresponding FISH analysis however showed gene amplifications in only 8% of cases, suggesting that other mechanisms apart from amplification might be involved in the overexpression of EGFR [19]. In another study, EGFR stromal overexpression further correlated positively with immunohistochemical stromal staining for p53, p16, Cyclin A, Cyclin E, Ki67 and c-kit [21]. Immunopositivity for EGFR in the stromal cells was detected in 19% of 58 phyllodes tumors (75% of all malignant tumors) [21]. Fluorescence in situ hybridization (FISH) showed whole-gene amplifications of EGFR in stromal cells in 15.8% of 58 phyllodes tumors and gene dosage PCR revealed intron 1 amplifications of EGFR in 41.8% of all phyllodes tumors [21]. The latter significantly correlated with tumor grade on the one hand, and EGFR overexpression on the other [21]. The presence of intron 1 amplifications also correlated with p16, p21 and p53 immunoreactivity [21]. Neither EGFR overexpression nor whole-gene amplification was observed in the control series of 167 fibroadenomas [21].

C-kit (CD117), a proto-oncogene encoding a tyrosine kinase membrane receptor protein, is another widely studied biomarker [16]. Progressive increases in c-kit immunohistochemical expression in stromal cells of benign (5% to 17% of cases) to malignant phyllodes (46% to 50% of cases) [16] show localization in subepithelial areas of stromal condensation [22]. C-kit has thus been considered another possible contributor to stromal proliferation in the phyllodes tumors [15, 16, 22, 23] presumably participating in the process of cell cycle progression, and synergistic with p53 protein, with which its

immunohistostaining has also been significantly correlated [15]. In one study, c-kit stromal expression correlated significantly with both phyllodes grade, as well as recurrent disease [15]. In another series, c-kit was also moderately to strongly positive in the epithelial component of benign phyllodes tumors, contrasting with the negative epithelial staining in the malignant ones [23], again implying autocrine/paracrine activation and interdependence of epithelium and stroma. Activating mutations of c-kit, like those in gastrointestinal stromal tumors (GIST), however, were absent [16, 23] Whether c-kit overexpression could be a potential basis for treatment of phyllodes tumors using tyrosine kinase inhibitor imatinib mesylate/Glivec, which is the treatment of choice in GISTs, is yet to be explored.

CD34 coexists with c-kit in GIST, and its possible coexistence with c-kit in phyllodes tumors was also investigated [24]. One study did not find significant correlation in the co-expression of CD34 and c-kit in phyllodes tumors [24]. CD34 is a type I transmembrane glycoprotein expressed on hemopoietic stem and progenitor cells, endothelial cells, and a subset of fibroblast and bone marrow progenitor cells, and is expressed in many mesenchymal tumors [24]. In the breast, CD34-expressing fibroblasts are considered CD34-positive dendritic cells in collagenous breast stroma [24]. CD34 was predominantly expressed in 6 of 7 benign phyllodes tumors in that series, comprising 50% to 90% of the stromal component [24]. In contrast, only 3 of 12 malignant tumors showed more than 10% stromal staining [24]. Inversely associated with CD34 expression in that same study was actin, which demonstrated myofibroblast differentiation in 8 of 12 malignant tumors and only one benign one [24]. Just like p53, Ki-67, and c-kit, the ability of CD34 and actin to predict outcome, however, was also described as questionable [24].

Not to be disregarded is the possible role of tumour vascularity promoting malignant behavior of phyllodes, concurring with studies using ET-1 as earlier mentioned. In one study, stromal microvessel density on CD31-stained slides of phyllodes tumors revealed significant increase in the number of vessels per high power field, between benign phyllodes tumour (mean range of 13.1) and borderline to

Molecular pathogenesis of breast phyllodes tumors

malignant phyllodes (mean ranges of 22.4 and 29.6, respectively) [25]. Similar to other reports correlating with phyllodes grade, there is also no significant difference between borderline and malignant phyllodes [25]. This was corroborated by related studies by the same author, using vascular endothelial growth factor (VEGF), also an angiogenic peptide that is mitogenic for endothelial cells [26]. Again, VEGF stromal expression increased significantly with increasing grade, correlating with mitotic rate and infiltrative margins [26], which was attributed to VEGF-recruited macrophages that further secrete VEGF and tumor growth cytokines [26].

Among proteins that can also potentially facilitate the invasive and metastatic potential of phyllodes tumors is heparan sulfate [27]. This protein is essential in intercellular and extracellular matrix adhesion, and is essential in stabilizing the binding of growth factors i.e. fibroblast growth factor, to their receptors [27]. In a recent study using 10E4 antibody that detects heparan sulfate in tissues, strong basement membrane and perithelial stromal expression was found in 11.2% of 232 phyllodes tumors, with strong accentuation immediately around the epithelial elements of the tumors [27]. 10E4 stromal expression showed significantly stronger staining intensity in phyllodes tumors with higher grades [27]. Interestingly, heparan sulfate stromal expression also correlated with p53 and c-kit stromal staining, and bcl2 epithelial staining, in this same study [27]. It has been previously reported that the regulation of bcl2, a well-known anti-apoptotic protein, is associated with alterations in p53, with which it has an inverse relationship, and their correlation suggested their involvement in malignant transformation in phyllodes tumors [27]. There was no association between 10E4 stromal positivity and any individual histological parameter of all the phyllodes grades [27]. Heparan sulfate now adds to the list of molecules cooperating in cell cycle progression that promotes stromal proliferation in phyllodes tumors.

CD10, also known as CALLA (common acute lymphoblastic leukemia antigen) is an example from the family of matrix metalloproteases which has also been reported to display similar increase in stromal expression correlating with increasing phyllodes grade [16], like the

majority of the stromal biomarkers. This marker, however, has not been extensively evaluated, although matrix metalloproteases, in general, function to degrade matrix adhesions rendering invasive and metastatic properties to any neoplasm.

Recurrence and metastasis

As has been mentioned, only a few studies showed biomarkers predicting recurrence in phyllodes tumors, and overall, no immunohistochemical nor molecular studies have shown consistently good correlation with the clinical outcome of phyllodes tumors. To date, the clinicopathologic parameter to predict recurrence, at best, is the margin status of the excision biopsy [2, 16], although even this alone, offers no guarantees. Several studies still state that there are widely excised phyllodes tumors with clear margins that nevertheless locally recur, or even metastasize, just as there are tumor-involved excision margins that have prolonged disease-free survival [2,14,16].

Current genetic updates

Taking all the above findings in consideration, a very recent comprehensive study of phyllodes tumors including recurrences was done, addressing the findings of benign versus borderline/malignant separations, intratumoral heterogeneity, mutations in genes frequently involved in various published immunohistochemical studies [14,15,16,17,21], and comparing genetic changes in primary phyllodes with their recurrent counterparts.

Using array CGH which identified new small regions of chromosomal gains and losses, and the Goldengate assay, which detected copy number changes and copy neutral loss of heterozygosity, the study again found frequent gain of 1q and deletion/copy-neutral LOH in chromosome 13 in borderline and malignant tumors [28]. Borderline tumors were genetically heterogeneous, although predominantly clustered with the benign ones [28]. CGH also detected new genetic changes of phyllodes tumors in their recurrent counterparts [28]. For example, although only 3 of 9 benign phyllodes showed histologic upgrade upon recurrence, 6 of 9 histologically benign tumors (67%) acquired new genetic

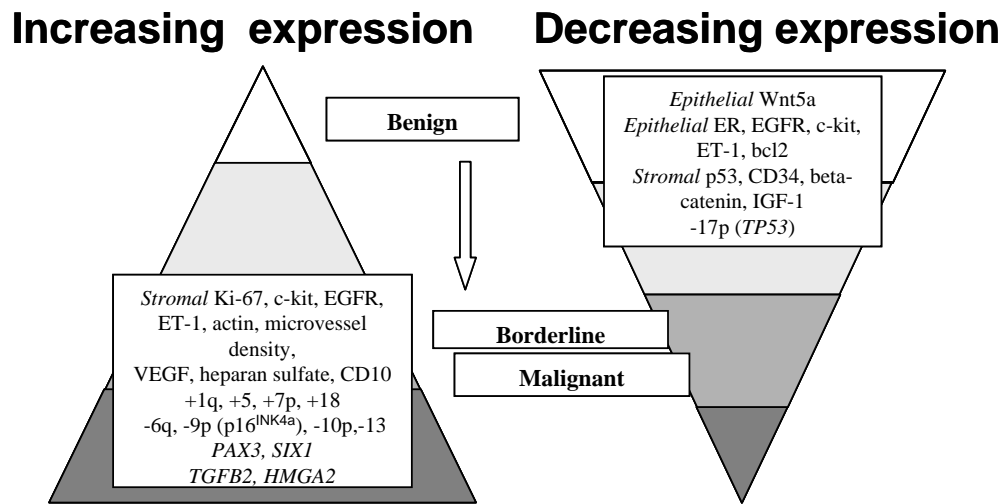


Figure 5: Correlations of immunohistochemical/genetic changes in the progression of phyllodes tumors. (Increase in shades of gray in the diagram levels depicts increase in grade)

changes that were associated with the borderline/malignant phenotype (+1q, +7p, -9p and -13) [28]. 7 of 10 (70%) borderline/malignant primary phyllodes also presented with new genetic changes in their recurrences [28]. The study found that in the borderline/malignant cases with 9p deletion, the deletion localized to 9p21.3, the site of p16INK4a [28]. In four other recurrent tumors, this deletion was not observed in the primary tumor [28]. Immunohistochemistry for p16INK4a showed positive stromal staining in almost half of 126 phyllodes tumors, with significant association with deletion of 9p [28]. The lack of correlation between genetic/immunohistochemical profiles with clinical outcome in most published studies was explained by this study's findings regarding intratumoral heterogeneity [28]. Genetic changes between random homogeneously cellular areas away from the epithelium, were mostly unidentical with those from the rest of the tumor [28]. Additional genetic changes were observed in microdissected areas of subepithelial stromal condensation, including a 1Mb deletion on 17p, a region containing TP53 [28]. This latter finding in a benign phyllodes was not seen in the malignant tumors however. It was thus uncertain if TP53 was the target in the 17p deletion, or if its mutation could be an early event [28].

Histologically benign phyllodes tumors that present with increasing genetic changes of the malignant phenotype, and the new deletions seen in recurrent tumors different from their primary, have now raised the hypothesis of sub-clones [28]. Sub-clones in phyllodes tumors possibly account for the discrepancy between clinical outcome and the morphologic features.

Lastly, a follow-up study by these same authors attempted to identify more specific genetic changes, particularly in borderline to malignant phyllodes tumors, apart from the earlier discovered 9p deletion localized to the site of p16INK4a. Using mRNA expression, they compared 12 benign with 11 borderline/malignant phyllodes tumors [29]. 162 genes were over-expressed in the borderline/malignant group; and functional annotation clustering revealed these genes were involved in development (40 genes), mitosis (24 genes), cell signalling (59 genes), cell cycle progression (23 genes), cell adhesion and ECM-receptor interaction (38 genes) [29]. Among these, PAX3 (chromosome 2q36) and SIX1 (chromosome 14) were the most significantly over-expressed in borderline/malignant phyllodes tumors [29]. PAX3 is known to be associated with rhabdomyosarcoma, and SIX1 has been shown to be associated with the metastatic behavior of this tumor [29]. The small foci of

Molecular pathogenesis of breast phyllodes tumors

PAX3 seen in 3 of 6 benign phyllodes tumors were interpreted as sub-clones with malignant potential [29]. SIX1, in contrast, was confined to the highly malignant phyllodes tumors in the study, and its expression was especially high in the tumor that metastasized [29]. TGFB2 (chromosome 1q) that is thought of as a tumor suppressor and a downstream target of PAX3, and HMGA2 (chromosome 12), frequently described in mesenchymal tumors, were also over-expressed predominantly in borderline/malignant phyllodes tumors [29]. Their over-expression in some benign tumors also ruled in their role in the transition from a benign to a malignant phyllodes tumor [29]. The mechanisms for these overexpressions, were however unclear, as there was no evidence of amplifications or translocations in these studied genes [29]. **Figure 5** shows a summation of the correlations of immunohistochemical/genetic changes in the progression of phyllodes tumors.

Summary

The histology of a biphasic pattern in phyllodes tumors displays its two key participants: the epithelium and the stroma, whereby the stroma may be dependent on the former until such time its proliferation overrides any epithelial influence. This epithelial-stromal interaction was explained by the stromal beta-catenin and epithelial Wnt pathway, which is among the more significant breakthroughs in the attempts to understand the molecular pathogenesis of phyllodes tumors. Past and present immunohistochemical studies would support this hypothesis, as they generally show parallel findings of significant increasing stromal expressions of biological markers portending aggressive behavior among benign, borderline to malignant categories, with inverse correlations with epithelial expressions. (See **Figure 5**) In general, however, histology nor immunohistochemistry alone could not accurately predict clinical outcome, and to date, the practical approach to prevent recurrence would still be widely clear surgical margins. Current studies have found that new genetic changes can develop within the same tumor, and in recurrent ones compared with their primaries, in keeping with phyllodes tumors' intratumoral heterogeneity. This acquisition of new mutations suggesting the possibility of sub-clones is the most current explanation of malignant progression

and recurrence of phyllodes tumors. More work needs to be done to further elucidate and define specific triggers of aggressive behavior.

Please address correspondences to: Dr Puay Hoon Tan, Department of Pathology, Singapore General Hospital, Outram Road, Singapore 169608, Tel: 65-63214874, Fax: 65 62226826, E-mail: tan.puay.hoon@sgh.com.sg

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Molecular pathogenesis of breast phyllodes tumors

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