HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL PROFILE OF BREAST PHYLLODES TUMOR

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Abstract. Phyllodes tumors arise from the epithelial and stromal components of the terminal duct-lobular unit. Because phyllodes tumors display a broad range of clinical and pathological behaviour, they should be regarded as a spectrum of fibroepithelial neoplasms rather than a single disease entity. This study represents a synthesis of the most recent data of the literature about breast phyllodes tumor, emphasized the histopathological, immunohistochemical and molecular characteristics of these neoplasms, with examples from our case-book records. Because of the potential of the phyllodes tumor to present recurrences and to metastasize and of the different histologic aspect, these were classified in three subgroups: benign, low grade malignant (borderline) and high grade malignant. The histologic distinction between benign and malignant phyllodes tumors is often difficult and arbitrary. The clinical behaviour of these fibroepithelial neoplasms is difficult to predict only after histologic aspect. Thus, the immunohistochemical characterization of these tumors, with the biological markers, will permit the elucidation of the pathogenic mechanisms, will facilitate the differential diagnosis with other types of lesions and will provide new alternatives of treatment. Because these markers can be identified either in malignant and benign phyllodes tumors, they can not be used separate of other investigations, for a proper classification of these lesions.

INTRODUCTION

Phyllodes tumor is a fibroepithelial tumor, histologic comparable with fibroadenoma, characterized by a more dense cellularity of the connective component. Phyllodes tumor is rare, representing 0,5-5% of all breast tumors (Rosen, 2006). The genesis of the phyllodes tumor is unknown. It is considered that this represents an evolutive possibility of fibroadenoma, translated by an exaggerate proliferation of the connective component (Rosen, 2001). The hormonal influences may have a role in appearance and evolution of this tumor. Because of the potential of the phyllodes tumor to present recurrences and to metastasize and of the different histologic aspect, these were classified in three subgroups: benign, low grade malignant (borderline) and high grade malignant (Rosen, 2006). The clinical behaviour of these fibroepithelial neoplasms is difficult to predict only after histologic aspect. Thus, the immunohistochemical characterisation of these tumors, with the biological markers, will permit the elucidation of the pathogenic mechanisms, will facilitate the differential diagnosis with other types of lesions and will provide new alternatives of treatment. Because these markers can be identified either in malignant and benign tumors, they can not be used separate of other investigations, for a good classification of these lesions (Rosen, 2006).

Macroscopic aspects of phyllodes tumors

Phyllodes tumor was for the first time completely described in 1838 by Johannes Muller. The term of ”cystosarcoma phyllodes” was chosen for describing the model of fern leaf and the flashy aspect of the lesion (Rosai, 2004). Among the numerous names given to this lesion, the only terms used everywhere today are ”periductal stromal tumor” and ”phyllodes tumor”. The term of *phyllodes tumor* is preffered for avoiding the diagnostic of sarcoma in benign variants. The diagnostic of phyllodes tumor must always included a subclassification as benign, low grade malignant (borderline) or high grade malignant. The distinction between these three subgroups is based on the histologic features of the tumor (Rosen, 2006).

The typical phyllodes tumor is round, relatively well circumscribed and firm. The limits of these tumors are not regular and they don’t have a real capsule. The phyllodes tumors which have microscopic invasive margins appear usually macroscopic circumscribed. In section,

phyllodes tumors appear firm, bosselated, gray-whitish (Rosen, 2001). There were noted degeneration focars, infarct and necrosis areas, with a gelatinous or haemorrhagic aspect. These features are more frequent in malignant tumors, but they can appear also in large benign phyllodes tumors. The cysts are rarely present. A less common variant of phyllodes tumor is that with an exaggerated cystic component, resulting a macroscopic aspect which can be difficult to differentiate from a cystic papilloma (Rosen, 2006).

Most of the phyllodes tumors are large, between 5-10 cm, but they can meassure less than 5 cm diameter. Thus, the diagnostic of phyllodes tumor is not based only on its dimensions. First, the diagnostic is confirmed by echography (which can observe, in large tumors, necrosis and lichefiation zones) and mammography (well circumscribed opacity, dense, which sometimes have calcifications) (Rosen, 2001; Pricop, 2003).

Histopathological features of phyllodes tumors

These tumors develop rather from periductal than intralobular stroma and usually contain scattered lobular elements. Most of the phyllodes tumors present a heterogenous histological aspect and only a small part describe the conventional tumor as having an exaggerated structure of an intracanalicular fibroadenoma, with a high cellularity stroma. In numerous cases, the intracanalicular aspect of the clefts is covered by the ductal hyperplasia (Rosen, 2006).

Microscopically, the two key diagnostic features are stromal hypercellularity and the presence of benign ductal elements. The tumors which have a fibroadenoma configuration have a cellular stroma, but does not have atypical features, that is why they are called *benign phyllodes tumors*. The glands dilated so much that in low power view, the tumor seems to have only stroma. The glands do not have atypical features. *The malignant phyllodes tumors* present also a loss of the relation stroma-glands, the stroma is hypercellular, the cells presenting a marked atypia and numerous mitoses (Rosen, 2001). The neoplasic stromal component seems to be monomorphous or polimorphous and can have an aspect of fibrosarcoma, malignant fibrous histiocytoma, lyposarcoma. Some authors consider that the borderline tumors must be regarded as low grade malignant tumors (they have a bigger recurrence tendency than the benign ones, but present a lower risk of distant metastasis than high grade malignant tumors) (Esposito et al, 2006). The tumor margins are invasive, which raise the risk of local recurrence. The benign phyllodes tumor is characterized by a tendency to local recurrence, but very rare gives distant metastasis. The malignant phyllodes tumor gives hematogenic metastases, mainly in lung, bone, central nervous system (Rosai, 2004).

There are several aspects which must be taken in consideration for the differentiation between fibroadenoma and benign phyllodes tumor. The phyllodes tumors are most frequently characterized by expansion and hypercellularity of the stromal component, comparative with fibroadenoma. In some phyllodes tumors, the stromal cellularity is more dense in adjacent zones of epithelial components (the periductal stroma) (Esposito et al, 2006). The mitotic activity can be high, while mitoses are virtually absent in fibroadenomas. There is a substantial group of phyllodes tumors in which the stromal zonal distribution can be absent.

The presence of elongated clefts lined by epithelium is a characteristic aspect associated to phyllodes tumors. Occasionally, these spaces are dilated, the adjacent stroma being condensed. The epithelial clefts can exist also in fibroadenomas. The intracanalicular structure of some fibroadenomas presents a superficial resemblance with the benign histoarchitectony of the phyllodes tumors, the difference between these two tumoral types being difficult to be made.

Histologically, the stroma in intracanalicular fibroadenomas tends to be hypocellular and uniform (Rosen, 2006).

The mixoid modifications appear in stroma of fibroadenoma and phyllodes tumors. These have the tendency to be homogenous distributed in fibroadenomas but can be focal and with degenerative aspects in phyllodes tumors. In phyllodes tumors appears pseudoangiomatous stromal hyperplasia (PASH), sometimes representing a proeminent feature of these lesions (Rosen, 2001). Rarely can be met stromal giant cells in phyllodes tumors with PASH. These cells can present lymphophagocytosis. The cells can express histiocytic immunomarkers, suggesting rather a hystiocytic histogenesis than myofibroblastic.

The stromal cellularity is frequent heterogenous in phyllodes tumors, with focars which are hard to distinguished by fibroadenomas. These areas can lead to the conclusion that phyllodes tumors arise from a fibroadenoma, when, actually, this represents an intrinsec aspect of a phyllodes tumor (Telli et al, 2007). This structural variability creates difficulties in classification of certain lesions obtained by fine needle aspiration biopsy or needle biopsy. Excision biopsy is necessary for the gradation of phyllodes tumors, determination based on stromal cellularity, mitotic activity and microscopic character of tumoral limits.

The subclassification of phyllodes tumors presumes, as already mentioned, three groups of lesions. It is important to distinguish the benign phyllodes tumors from low grade malignant ones, because the former don’t metastasize and have a low risk of recurrence (Yabuuchi et al, 2006). The low grade malignant tumors present earlier local recurrences and often with a high grade malignancy (Rosen, 2006).

The benign phyllodes tumor is characterize by few or absent mitoses (1-2 mitoses/x10 PF). In most tumors is observed a marked increase in cellularity with a low to moderate cellular pleomorfism. The stromal expansion is often uniform at the entire lesion, but it can be also heterogenous. The degree of epithelial proliferation coresponds usually with the stromal aspect (figure 1). Epithelial hyperplasia is not evident in usual benign phyllodes tumors, but sometimes can be pronounced. The tumoral limits are usually well delimitated. The lipomatous or bony metaplasia can appear in the stroma of a benign phyllodes tumor. It can be present also multinucleated stromal cells with hyperchromatic nuclei (Rosen, 2001).

At the other extreme, a malignant phyllodes tumor presents a high grade of stromal hypercelularity, in the majority of cases resulting in a separation of epithelial elements with proliferative activity in the stroma (often more than 5 mitoses/x10 PF) and tumoral invasive margins. The stromal cellular pleomorfism appears often in these lesions (figures 3 and 4). Rarely, the stroma contains sarcomatous heterologous elements, like angiosarcoma, lyposarcoma, chondrosarcoma, myosarcoma or osteosarcoma (Confavreux et al, 2006; Fernandez-Aguilar, 2007; Hsu et al, 2007; Ribeiro-Silva, 2006; Uriev et al, 2006).

The borderline or low malignant phyllodes tumors present microscopic invasive margins, a media of 2-5 mitoses/x10 PF and moderate stromal cellularity (figure 2), often heterogenous distributed in the middle of hypocellular areas. In most of these lesions, the fusiform cells stroma reminds the fibromatosis from low grade fibrosarcomas (Houssami et al, 2007).

A lot of phyllodes tumors present epithelial hyperplasia, often represented by a variable increasing in the thickness of cuboidal or cilindrical epithelium which lines the glandular spaces. The increase in thickness includes also the hyperplasia of myoepithelial cells, which can progress focal or diffuse toward papillary or cribriform hyperplasia (Rosen, 2006). The atypical epithelial hyperplasia is sometimes extreme, leading to a diagnosis of intraductal carcinoma (Merck et al,

2006). The character of phyllodes tumor of the lesion can be unobserved if the stromal component is interpreted as reactive and not as an intrinsec part of the neoplasm.

The squamous metaplasia of the ductal epithelium, which appears in benign and malignant tumors, is met in about 10% of phyllodes tumors (Sugie et al, 2007). The aspiration of a cystic area of squamous metaplasia can lead to a wrong diagnosis of squamous cyst. It war reported also apocrine metaplasia of the epithelium of phyllodes tumors (Rosen, 2006). The lobules are occasionally included or formed in phyllodes tumors, these could presenting proliferative modifications, including sclerosis adenosis. The presence of the lobules can lead to a misdiagnostic of fibroadenoma, mainly when it is a lobular hyperplasia and the stromal cellularity is not very increased.

At **ultrastructural** level, the stroma of phyllodes tumor is composed of cells with fibroblastic and myofibroblastic aspects, which remind of normal cellular components of the mammary gland stroma. There were described electron-dense cytoplasmic bodies, sometimes with crescent shape. It seems that these structures have a lisosomal origin, being much more frequent in malignant tumors. The fibroblasts present ocasionally tight jonctions. The normal fibroblasts were sometimes met among neoplastic cells. The intermediar filaments and dense bodies were observed in myofibroblastic cells (Rosen, 2001). The electron microscopy doesn't present unusual aspects in the epithelial component of phyllodes tumor.

The cytologic diagnostic of a phyllodes tumor can be suggested by an aspirate which have a typical epithelial component for a fibroepithelial neoplasm, with bipolar stromal cells. Characteristic for phyllodes tumor are rather stromal cells with cytoplasm than bipolar naked nuclei (Rosen, 2006). The stromal cell fragments are useful in distinguish the phyllodes tumors from fibroadenomas. The aspiration cytology represents sometimes an uncertain diagnostic procedure for a phyllodes tumor. The lesions with a marked epithelial hyperplasia can lead to an aspirate in which the stromal component is masked, this situation creating an erroneous diagnostic of carcinoma or fibroadenoma, if the specimen obtained from a heterogenous tumor presents an epithelium without modifications and isolated stromal cells. The aspirate from a malignant phyllodes tumor contains probably stromal cell fragments composed of atypical cells and possible mitotic figures (Istvanic et al, 2007). The stromal fragments with adipous differentiation can be present in a cytologic specimen from a phyllodes tumor with lyposarcomatous differentiation (Uriev et al, 2006).

The differential diagnosis of phyllodes tumor is made with juvenile fibroadenoma, with breast carcinosarcoma (extremely rare malignant tumor, composed of an independent byphasic malignant proliferation, stromal and epithelial) and with metaplastic mammary carcinoma (a variant of infiltrative ductal carcinoma, in which the epithelial component, unlike phyllodes tumor, is malignant, but appear areas of squamous, cartilaginous metaplasia, and even sarcomatous transformation of the stroma) (Cserni et al, 2006 ; Fajdic et al, 2007).

Immunohistochemical and molecular features of phyllodes tumor

The stroma of phyllodes tumor is vimentin positive (figure 5). The reactivity of actin, CD34, and desmin is present in a variable proportion of cases which present mixoid or pseudoangiomatous stromal differentiation of myofibroblasts (Aranda et al, 1994). The stromal cells are not S100 positive. The excessive perivascular deposition of collagen type IV was observed in the stroma of malignant phyllodes tumors, but not in the benign ones, a similar aspect being observed in noncystosarcomatous sarcomas (Rosai, 2001). The immunohistochemical distribution of Ki-67 antigen, detected with MIB1 antibody is correlated

with the difference between benign and malignant phyllodes tumor (Chan et al, 2004). The MIB1 index of tumoral stromal cellularity as well as the proportion of MIB 1 positive cells were significantly bigger in malignant than benign phyllodes tumors. A much smaller difference was observed between benign phyllodes tumors and fibroadenomas MIB1 index. The differentiated expresion of p53 was observed also in fibroepithelial tumors (Chan et al, 2004). The higher activity was in malignant phyllodes tumors, especially in periepithelial stroma (figure 6).

The phyllodes tumors contain a much more concentration of endothelin-1 than fibroadenomas (Tse et al, 2007). This vasoconstrictor peptid stimulates the DNA synthesis in vascular smooth muscle cells and in mammary stromal cells. Immunohistochemical studies revealed that endothelin-1 was located in the epithelium of phyllodes tumor, but it was absent in the stromal cells of the same tumor (Tse et al, 2007). These observations suggest that endothelin-1, elaborated by the epithelial component of phyllodes tumor, can have a paracrin function in stimulation of stromal cells proliferation. The conventional histologic observations and the morphometric studies revealed that the mitoses tend to be more frequent in the stroma from epithelial proximity of phyllodes tumors, which is, again, in concordance with paracrin function of the epithelium. Evidences of paracrine interactions between epithelial and stromal components in phyllodes tumors are justified by the coexistence of platelet derived growth factor (PDGF) and of beta receptor PDGF in both tisular compartments, too (Feakins et al, 2000). The alelic unbalance was detected both at epithelial and stromal levels in phyllodes tumors, suggesting that both components can contribute to the neoplastic process in certain conditions.

Tenascin represents an extracellular matrix glycoprotein which inhibites the cells interaction with other cells and stroma. The immunohistochemical studies revealed that tenascin is distributed in a limited subepithelial zone from normal stromal mammary tissue and fibroadenomas and more difuse in stroma of phyllodes tumors (Arihiro et al, 2000).

It was studied the expression of C-kit, a proto-oncogene which codifies the tirosin-kinase receptor, in phyllodes tumors stroma. It was observed a significant higher frequency of C-kit immunoreactivity in high grade malignant phyllodes tumors comparative with benign ones (Tse et al, 2004; Carvalho et al, 2004). The detection of C-kit expression in ceratin phyllodes tumors can be useful in the treatment of these tumors with drugs that inhibit the receptors for tirosin kinase (Tse, 2004).

It was reported also the expression of vascular endothelial growth factor (VEGF), which is a grade indicator of the phyllodes tumor. It was identified a much more intense reactivity in the stroma of malignant phyllodes tumors comparative with benign ones (Tse et al, 2004).

The reactivity of CD10 was signbificant more ferquent in the stroma of high grade malignant phyllodes tumors comparative with benign, borderline tumors or fibroadenomas. The expression of CD10 tends to be stronger in subepithelial regions of the tumor (Tse et al, 2005).

Because these markers can be identified in both malignant and benign tumors and can be absent in certain malignant tumors, they can not be used alone, as a base of classification of these lesions. The immunohistochemical expression of these molecules may be of prognostic relevance in phyllodes tumors and can provide a highlight of the molecular pathway in carcinogenesis and progression of breast phyllodes tumours.

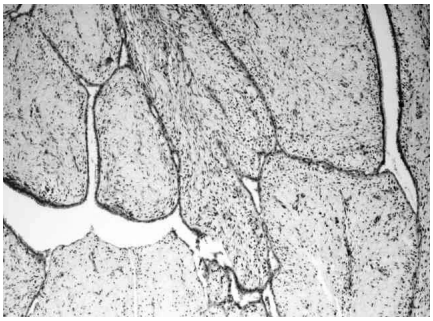

Figure 1. Benign phyllodes tumor, numerous epithelial clefts, high stromal cellularity, HE x 5

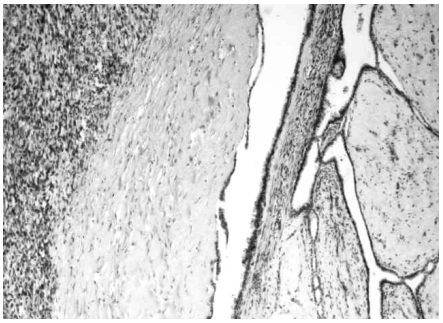

Figure 2. Low grade malignant phyllodes tumor (left), benign area (right), HE x 10

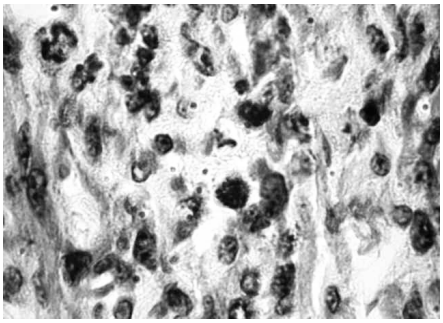

Figure 3. High grade malignant phyllodes tumor atypical cells, mitosis, HE x 40

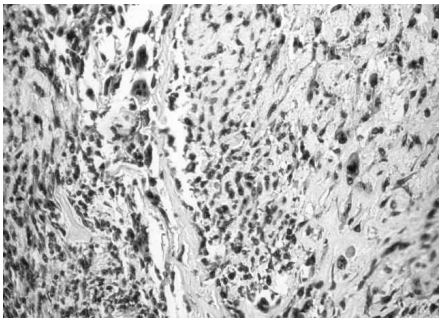

Figure 4. High grade malignant phyllodes tumor, atypical cells, multinucleated giant cells (superior and central), mixoid modifications (right), HE x 20

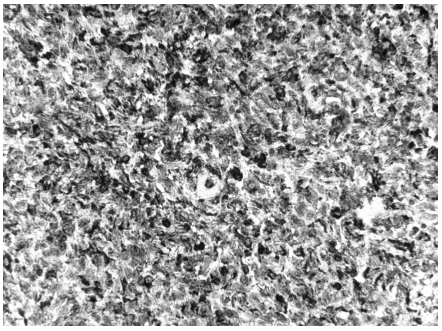

Figure 5. Phyllodes tumor, immunopositivity for vimentin, x 10

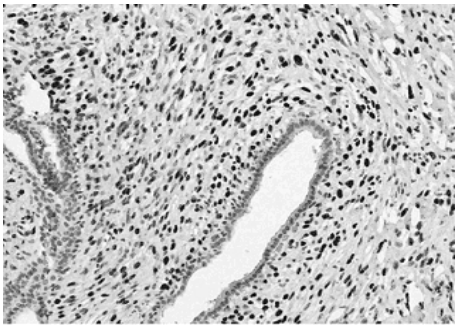

Figure 6. Phyllodes tumor, immunostaining for p53 in stromal cells, x 10

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