HELLENIC SOCIETY OF PATHOLOGY
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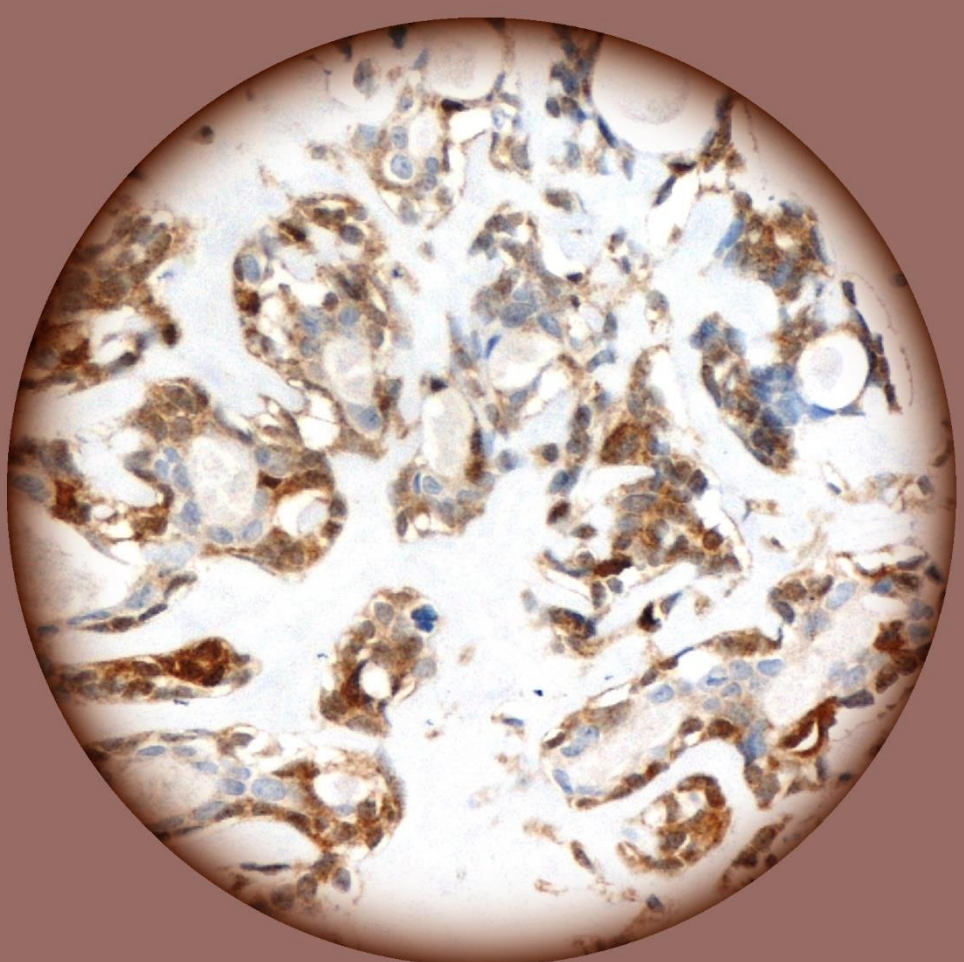
1964

JOURNAL OF SURGICAL AND MOLECULAR PATHOLOGY

VOLUME 2| ISSUE 1



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Maspin immunoreactivity in salivary pleomorphic adenomas

Ioannis Bellos[1], Pavlos Maragkoudakis[2], Evangelos Plantzas[2], Lambros Korogiannis[1], Dimitrios Kandiloros[2] and Andreas C. Lazaris[1]

Abstract

Aim: Maspin is a serine protease inhibitor with possible suppressive effects over tumor progression. The aim of this study was to describe maspin expression pattern in pleomorphic adenomas of the salivary glands, in order to investigate the potential role of this molecule in the benign nature of this neoplasm.

Materials and Methods: Immunohistochemistry staining was applied in a sample of 120 pleomorphic adenomas and also in 10 high grade adenoid cystic carcinomas, which served as a control group of malignant biphasic tumors.

Results: All pleomorphic adenomas exhibited a strong selective positivity of their myoepithelial component, while luminal cells were invariably not immunostained. On the contrary, all adenoid cystic carcinomas presented a negative profile for maspin, predominantly in the highly malignant areas with a solid pattern of development.

Conclusion: It is suggested that maspin expressed selectively by myoepithelial cells of pleomorphic adenomas, may function as a tumor-suppressor factor in salivary neoplasia.

Introduction

Maspin (mammary serine protease inhibitor) is a member of the serpin (serine protease inhibitor) super-family with tumor-suppressor properties under investigation. Its function is arguably characterized by the ability to hinder tumor growth and metastasis via an increase in cell adhesion, inhibiting migration and angiogenesis. It is localized in the nucleus, the cytoplasm or it is secreted, while the amount of its expression and its subcellular localization exerts significant prognostic influence in a variety of neoplasms [1].

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The aim of this study is to describe the distribution of maspin expression in pleomorphic adenoma of the salivary glands and to speculate the role of this molecule in the nature of this tumor.

Materials and Methods

The sample was derived from the records of the First Department of Pathology, Medical School, National and Kapodistrian University of Athens, Greece with the approval of the Ethics Committee of this institution. It consisted of 120 pleomorphic adenomas of the parotid gland; 10 high grade adenoid cystic carcinomas of minor salivary gland origin were also evaluated as a group of malignant tumors with a biphasic nature, similar to that of pleomorphic adenomas. Four-micrometer thick sections were obtained from formalin fixed and paraffin embedded specimens. Antigen retrieval was performed (DAKO, EDTA, pH=8) and anti-maspin antibody were applied (EAW24, dilution 1:10). Diaminobenzidine was used as a chromogen and Gill's hematoxylin as a counterstain. Sections from prostate tissue served as positive controls. Maspin staining intensity was generally quite strong probably due to uniform conditions of fixation and non-oldness of all specimens. In the vast majority of benign tumors, the myoepithelial component was positive at percentages >80% (Table 1). Anti-muscle actin antibody (HHF-35) was utilized as an immunomarker of the myoepithelium at specific sites,where identification of these cells was not conspicuous morphologically.

Table 1. Overview of maspin cytoplasmic immunostaining findings.

Maspin immunopositivity	Type of sample	
	Pleomorphic adenomas (n=120)	High-grade Adenoid cystic carcinomas (n=10)
≥ 80% of myoepithelial cells	103 (85.8%)	-
60-80% of myoepithelial cells	17 (14.2%)	-
Up to 1% of myoepithelial cells	-	8 (80%)
Totally negative	-	2 (20%)

Results

All cases of pleomorphic adenoma exhibited selective maspin positivity at their myoepithelial component, the latter often morphologically detectable as a basal layer in glandular structures; immunostaining presented a mainly cytoplasmic staining pattern; combined cytoplasmic and nuclear staining was clearly observed in a minority of pleomorphic adenomas (17 out of 120 cases). On the contrary, identifiable luminal cells were not immunostained, as shown in figure 1. Adenoid cystic carcinomas (ADCCs) with predominantly solid architecture served as a malignant group of salivary tumors also of a biphasic nature (i.e. epithelial-myoepithelial, like pleomorphic adenomas) and presented a diffuse, almost totally negative immunohistochemical profile (except for few immunopositive cells in two cases), always with prominent complete loss of maspin expression at their highly malignant areas with a solid pattern. Thus, as shown in Table 1, the difference in maspin expression between pleomorphic adenomas and ADCCs is evident, as the former presented cytoplasmic immunopositivity in at least 60% of myoepithelial cells, while the latter were totally negative or positive in ≤1% of myoepithelial cells.

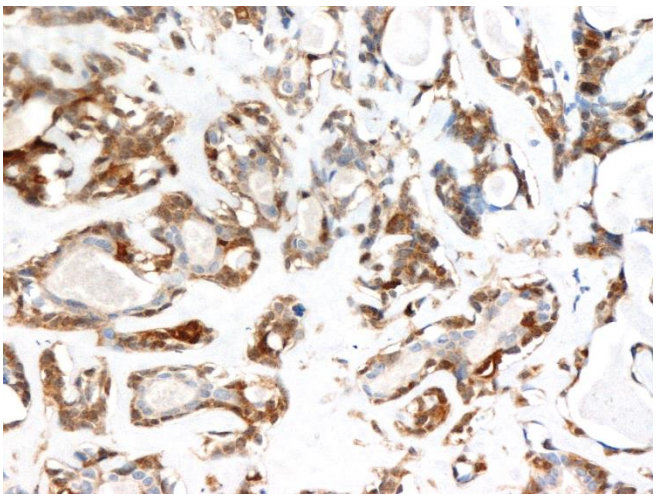

Figure 1. Evident selective maspin immuno-expression in the myoepithelial component of a pleomorphic adenoma (Immunoperoxidase stain, ×250)

Discussion

In the present study, immunohistochemistry has revealed a specific myoepithelial expression of maspin in benign biphasic salivary tumors (i.e., pleomorphic adenomas) and not in one of their malignant counterparts. It is of interest that all ADCCs examined in the present study were practically maspin- immunonegative, despite the presence of separate myoepithelial cells among the neoplastic cell solid population, as verified by their HHF-35 imunoreactivity. Therefore, it is suggested that maspin- positive myoepithelial cells play a pivotal role to the benign nature of pleomorphic adenomas.

It has been shown in the literature that carcinomas with myoepithelial differentiation, regardless of the amount of myoepithelial cells, are associated with a significantly lower vascular density [2]. As shown in mammary ductal carcinoma in situ (DCIS), myoepithelial maspin has the ability to inhibit invasion by separating ductal cells from the stromal angiogenesis [3].However, despite their natural tumor-suppressor function, myoepithelial cells in pleomorphic adenoma have been reported to exhibit an increased expression of Vascular Endothelial Growth Factor (VEGF) and of its receptors, a fact that is probably attributed to the diversity of characteristics of this particular tumor's stroma [4]. In agreement with the above finding, a strong positivity of pleomorphic adenomas for the panendothelial CD34 marker has been reported [5], reflecting high vascularity, especially in their cellular-rich areas. Nevertheless, these benign tumors have been reported to show minimal CD105 staining, demonstrating low relevant density of newly formed microvessels and, consequently, no malignant potential [6]. On the other hand, carcinoma ex pleomorphic adenoma, which is characterized by intense maspin expression in its early stages but a complete loss of maspin in invasive carcinomatous areas[6], is known to be triggered by an angiogenic switch with a considerable increase of neovascularization evidenced by CD105 staining [7].Consequently, combining our finding with those of previous studies, it can be proposed that myoepithelial maspin expression in pleomorphic adenoma potentially constitutes a tumor-suppressor factor, which may exert remarkable negative control over the tumor's density of newly formed microvessels; further comparative investigation of angiogenesis markers is of course needed to support this suggestion.

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Hypoxia inducible factor-1α (HIF-1α) and matrix metalloproteinase 9 (MMP-9) expression in invasive breast cancer

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Abstract

Aim: Hypoxia has a key role in cancer progression and metastasis. Low tissue oxygen availability induces the activity of HIFs (Hypoxia-Inducible Factors) to up-regulate a panel of invasion related genes, in a plethora of solid tumors. MMPs (matrix metalloproteinases) are enzymes produced from normal and cancerous cells with the ability to degrade extracellular stroma, priming invasion. The aim of this study was to identify possible gene and protein associations between HIF-1α and MMP-9 in invasive breast cancer.

Materials and Methods: A total of 96 FFPE (Formalin-Fixed Paraffin-Embedded) breast cancer samples were evaluated for HIF-1α and MMP-9 protein expression. Publicly available gene expression datasets for breast cancer were analyzed for HIF-1a and MMP-9 gene expression. Results were correlated with clinicopathological parameters and patient clinical outcome.

Results: MMP-9 and HIF-1α showed a positive correlation both at the mRNA ($p<0.001$) and protein ($p=0.01$) expression levels, in samples from breast cancer patients. This correlation was specific because HIF-2b, another member of the HIF family, presented with a negative correlation with MMP-9 gene expression. Furthermore, mRNA and protein expression of MMP-9 had a negative correlation ($p<0.01$) with relapse-free survival (RFS) and a positive correlation ($p<0.01$) with lymph node status (N1), respectively. In addition, patients with high mRNA expression of HIF-1α had significantly ($p<0.001$) shorter OS and RFS than did patients with low levels of HIF-1a.

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Conclusions: HIF-1α and MMP-9 co-expression may represent a novel marker of worse clinical outcome in breast cancer patients, predicting increased metastatic capacity of cancer cells.

Introduction

Hypoxia is a major driving force of tumor progression and cancer metastasis [1]. The presence of tumor hypoxia is correlated with poor overall survival and increased metastatic incidence in patients with various solid tumor types, including breast cancer [2,3,4,5]. Hypoxic cancer cells tend to physically move toward an oxygen rich environment, being selected for higher invasiveness and metastatic capacity, promoting malignant progression [6]. The molecular sensor of cellular hypoxia is a family of transcription factors (Hypoxia Inducible Factors, HIFs) responsive to reduced intracellular oxygen availability. Hypoxia inhibits HIF degradation and promotes their transcriptional activity. Over 1000 HIF target genes have been identified so far, and amongst them HIF-1α regulates the expression of several key tumor progression genes (e.g CXCR4, RIOK3, LOX) [7,8]. Immunohistochemical studies on breast tumor samples have linked increased HIF-1α protein levels with increased risk of metastasis and mortality in unselected breast cancer patients [9,10,11].

In more detail, hypoxia-induced invasion and metastasis involves several steps where cancer cells must cross numerous extracellular matrix (ECM) barriers, such as the epithelial basement membrane and blood vessels or lymphatics. MMPs (matrix metalloproteinases) are a large family of metal-binding proteinases with enzymatic activity against all components of the ECM and basement membranes. MMP-9 has the ability of degrading extracellular matrix and directly regulates angiogenesis by increasing the bioavailability of the pro-angiogenic factor VEGF. In addition, the formation of experimental lung metastases in mice is reduced by downregulation of MMP-9 in cancer cells [12], and is also reduced in the MMP-2- and MMP-9-null mice as compared with wild-type mice [13].

However, there are limited data concerning possible molecular interactions between the two proteins in breast cancer progression. In the present study we investigated HIF-1α and MMP-9 immunohistochemical expression in invasive breast cancer tissue samples and its correlation with tumor clinicopathological parameters. Furthermore, we analyzed publicly available mRNA expression datasets for possible HIF-1a and MMP-9 gene associations.

Material and Methods

Tissue specimens

Formalin-fixed paraffin-embedded postsurgical specimens from human breast carcinomas were retrieved from the archives of the Department of Pathology, University Hospital of Patras, Greece. There were 96 FFPE (formalin fixed paraffin embedded) samples of invasive breast cancer. Data for breast cancer prognostic factors (HER2, proliferation index Ki67 and p53 protein) and receptor expression (ER, PR) were collected from the original pathology reports (Figure 1.A). This study was conducted according to the principles laid down by the Declaration of Helsinki.

Clinicopathological characteristics.		
Mean age at diagnosis (years)	56	Range: 32 – 80
Median tumor size (cm)	2.8	Range: 0.1 – 9.0
TNM stage n (%)	I IIa IIb IIIa IIIb IIIc IV	13(13.5) 18(18.8) 24(25) 8(8.3) 4(4.2) 12(12.5) 17(17.7)
Lymph node status, n (%)	Positive Negative	64/96 (66.7) 32/96 (33.3)
Tumour grade, n (%)	1 2 3	9/96 (9.4) 63/96 (65.6) 24/96 (24)
ER status, *n* (%)*	Positive Negative	74/96 (77.1) 22/96 (22.9)
PR status, *n* (%)*	Positive Negative	66/96 (68.8) 30/96 (31.3)
hER2 / c-erbB2 status, *n* (%)*	Positive Negative	31/96 (32.3) 65/96 (67.7)
p53 status, *n* (%)*	Positive Negative	47/96 (49) 49/96 (51)
Ki67 status, *n* (%)*	Low Intermediate High	57/96 (59.4%) 18/96 (18.8%) 21/96 (21.9%)

Figure 1.A Clinicopathological parameters of the in-house breast cancer patient cohort

Immunohistochemical evaluation

Immunohistochemical analysis was carried out as previously described [14]. Briefly, the anti-MMP-9 (Novocastra) or the anti-HIF-1α (R&D Systems) primary antibodies (dilutions 1:15 and 1:30, respectively) and Envision Detection System (DAKO, Hamburg, Germany) were used for visualization. For each section an assessment was made both of staining intensity and of percentage of cells staining in separate scales from 1 to 3. Then for each section the two scores were added to obtain the scores as 0, 1, 2, 3, 4, 5 or 6. Tumors having a final staining score of 1, 2 or 3 were lumped to a low expression group and 4, 5 or 6 to a high expression group.

Gene expression data and statistical analysis

Gene expression datasets were downloaded from The Cancer Genome Atlas (TCGA) portal (cancergenome.nih.gov/). Breast cancer survival analysis was performed through KM-plotter as described in Gyorffy et al. [15]. Statistical analysis was performed with SPSS, v15.0 for Windows (SPSS Inc., Chicago, IL, USA). $P<0.05$ was considered statistically significant.

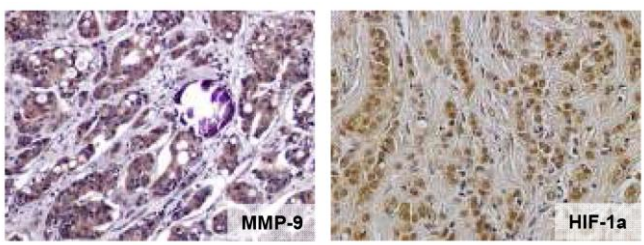

Figure 1.B Expression of MMP-9 and HIF-1α in breast cancer cells at 40x magnification

Results

1. Immunoexpression of HIF-1α and MMP-9 in breast carcinomas and adjacent normal tissue.

Immunohistochemical staining for HIF-1α and MMP-9 was performed on 96 and 95 tumors and uninvolved adjacent breast tissue, respectively. The normal breast tissue did not express either of the molecules. In breast cancer tissue, MMP-9 showed high cytoplasmic expression in 64/96 (66.6%) of cases, low in 22/96 (22.9%) and negative in 10/96 (10.5%) (Figure 1.B). There was also rare weak expression in endothelial cells and fibroblasts of cancer stroma. Positive HIF-1α expression was identified in cancer cells, while cancer stroma was negative (Figure 1.B). High expression of HIF-1α was found in 64/96 (66.6%) of cases, low in 18/96 (18.8%) and negative in 14/96 (14.6%). Importantly, there was a statistically significant correlation between expression of HIF-1α and MMP-9 in cancer cells ($r=0.264$, $p=0.01$). To further test a possible co-expression of HIF-1α and MMP-9 in breast cancer

at the mRNA level, we employed TCGA publicly available data from breast cancer patients to evaluate a potential correlation between HIF-1a and MMP-9 gene expression. We analyzed mRNA expression (RNA Seq V2 RSEM) data for HIF-1a and MMP-9 genes from an unselected cohort of 971 breast cancer patients with available data for both genes under study. HIF-1a mRNA showed a statistically significant positive correlation ($p<0.001$) with the mRNA expression of MMP-9, while there was a negative correlation between HIF-2b, another member of the hypoxia-inducible factor family and MMP-9 (Figure 1.C, upper panel). In addition, HIF-1a expression correlated positively with MMP-9 in estrogen receptor positive (ER+) cases ($p<0.01$), but not with ER- cases ($p=0.296$) from the same TCGA breast cancer patient cohort (Figure 1.C, lower panel and data not shown).

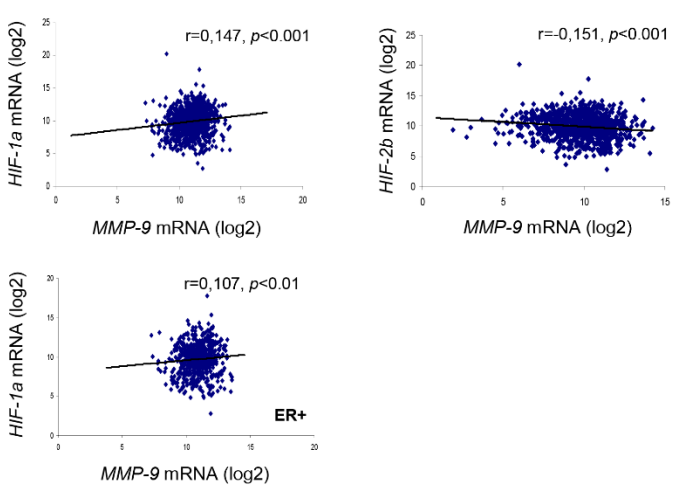

Figure 1.C HIF-1α and MMP-9 co-expression at the mRNA level (upper left panel), HIF-1β and MMP-9 co-expression at the mRNA level (upper right panel) and HIF-1β and MMP-9 co-expression at the mRNA level in ER+ breast cancer patients (lower left panel)

2. Association of HIF-1α and MMP-9 with clinicopathological data and clinical outcome.

In order to examine the correlation between expression patterns of MMP-9 and HIF-1α with clinicopathological parameters, statistical analysis was performed on both in-house cases with IHC expression data and publicly available datasets of breast cancer patients. The results showed that MMP-9 IHC expression correlated with a marginal significance ($p=0.046$) with the TNM stage of breast tumors and lymph node status ($p<0.01$). To assess in an extended cohort, possible correlation of HIF-1α and MMP-9 with breast cancer patient clinical outcomes, we employed microarray data of breast tumors from 4,142 patients [15]. 3,554 patients (all breast tumor types with valid probe sets) were analyzed for relapse-free survival (RFS) in conjunction to HIF1-a and MMP-9 gene expression. Those with high levels of HIF1-a and MMP-9 mRNA expression (the median value was used as the cutoff for low and high expression) had significantly shorter relapse-free survival than did patients with low levels of HIF-1a ($p<0.001$) and MMP-9 ($p<0.01$) (Figure 2.A and B, respectively). In addition, in the same dataset HIF1-a expression had an inverse correlation ($p<0.001$) with overall patient survival (OS) (Figure 2.C), while MMP-9 expression was inversely correlated ($p<0.01$) with OS in the estrogen receptor negative (ER-) group of patients (n=293) (Figure 2.D).

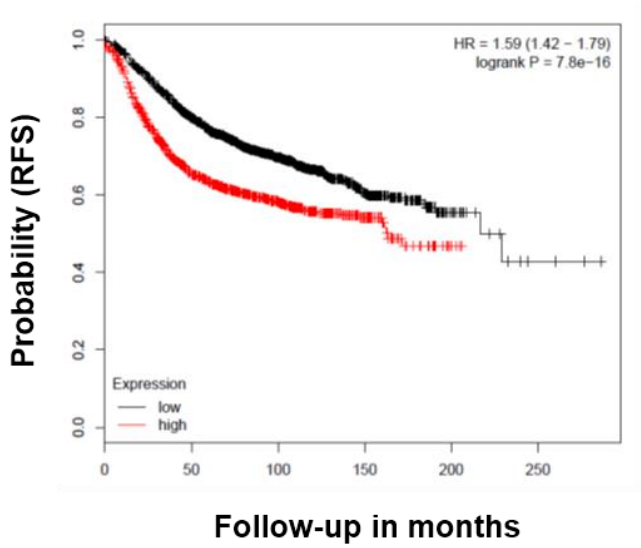

Figure 2.A Kaplan-Meier curves of relapse-free survival (RFS) for HIF-1a gene expression. Statistical significance was determined by log-rank test.

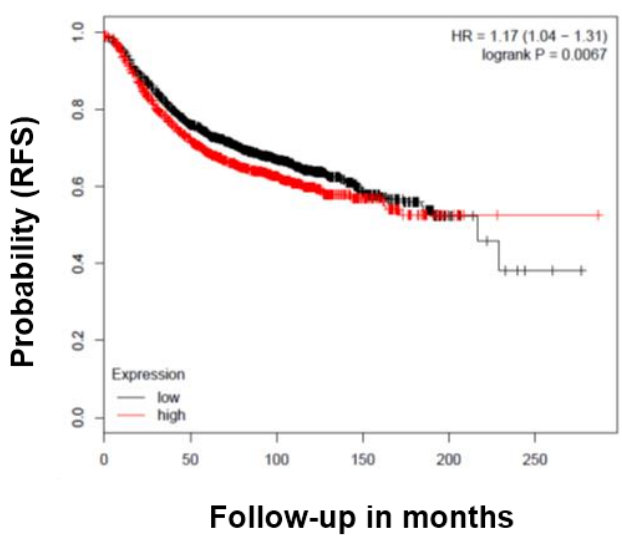

Figure 2.B Kaplan-Meier curves of relapse-free survival (RFS) for MMP-9 *gene expression. Statistical significance was determined by log-rank test.*

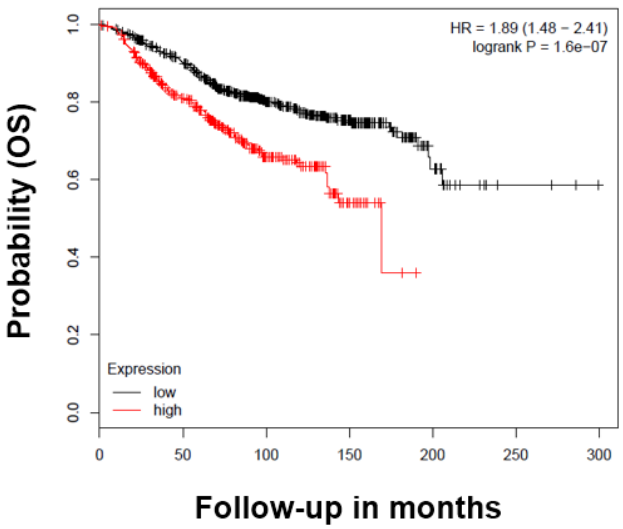

Figure 2.C Overall survival (OS) for HIF-1a in the whole patient cohort. Statistical significance was determined by log-rank test.

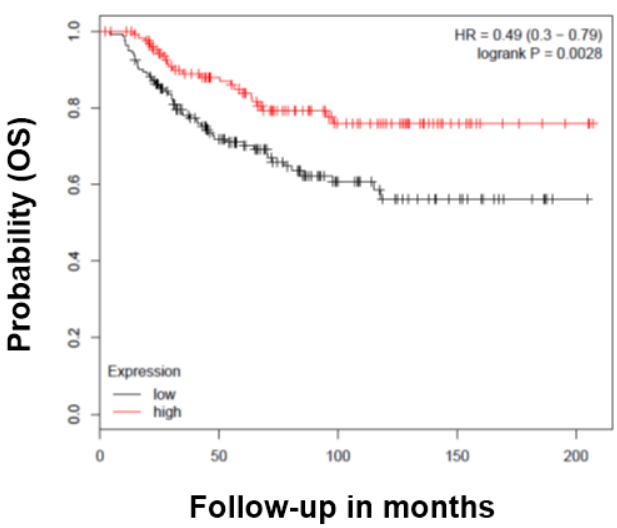

Figure 2.D Overall survival (OS) for MMP-9 in ER- breast cancer patients.

Discussion

The crucial role of hypoxia in tumor pathophysiology and metastasis is well documented [1]. However, the underlying mechanisms are still not fully elucidated. The metastatic cascade involves migration, invasion,

adhesion and survival of tumor cells and these cellular "states" constitute rate limiting steps in the developing metastases [4]. Hypoxia induces the invasive capacity of tumor cells, and through the activation of hypoxia-inducible transcription factors, a panel of invasion-promoting genes is upregulated. Important barrier to cell movement is the extracellular matrix which can be degraded by the enzymatic activity of matrix metalloproteinases, produced by cancer cells. MMP activity is a well-established hallmark of invasive phenotypes in many solid tumors and in breast cancer.

In the present study, MMP-9 protein expression was positively correlated with lymph node metastases, while high mRNA expression was correlated with shorter RFS and reduced OS in ER negative patients. At the same time, mRNA expression levels of HIF-1α, a major oxygen homeostasis regulator and endogenous marker of hypoxia widely studied in breast cancer, were inversely correlated with RFS and OS in the same cohort of breast cancer patients.

Moreover, immunohistochemical expression of HIF-1α, revealed a positive correlation with MMP-9 expression in breast cancer cells. This positive correlation was also identified at the mRNA level, after analyzing publicly available RNA sequencing data from a cohort of 971 breast cancer patients. These data provide a possible link between hypoxia, HIF-1α activation and cancer cell invasion, through the up-regulation of MMP-9 in breast cancer.

Conclusions

The results presented in this study provide preliminary evidence for a possible co-expression of HIF-1α and MMP-9 in breast cancer, while underscores the clinical significance of HIF-1a and MMP-9 gene expression in conjunction to RFS and OS. However, immunohistochemistry for HIF-1α and MMP-9 only detects protein expression without determining functional implications in cancer biology, while mRNA expression does not fully reflect protein levels. Therefore, larger scale functional studies are needed in order to clarify the biological significance of different HIF-1α and MMP-9 expression patterns and elucidate whether there is a HIF-1α-induced MMP-9 expression pathway in breast cancer.

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Lymphocytic Vasculitis : Classification of 127 cases

Özay Gököz[1] and Çisel Aydın[1]

Abstract

Aim: Lymphocytic vasculitis is a morphological term which includes clinically heterogenous diseases like connective tissue disease, infection, lichenoid diseases, drug reaction, Behçet's disease, superficial thrombophlebitis and leukemic vasculitis. There are three forms of lymphocytic vasculitis : angiodestructive form, lichenoid lymphocytic vasculitis and lymphocytic endovasculitis. There is a need to classify the diseases with the pathologic diagnosis of lymphocytic vasculitis.

Materials and Methods: In this study, 127 cases of lymphocytic vasculitis diagnosed between 2001-2013 were classified according to the clinical setting. The histopathological diagnosis was given to the lesions with angiotropism/diapedesis by lymphocytes, erythrocyte extravasation and swelling of endothelial cells, with/without fibrinoid necrosis of the vessel wall.

Results: Clinical diagnoses were collagen vascular disease (CVD, n=25; including 6 dermatomyositis, 2 chillblain lupus, 2 morphea), urticarial/leukocytoclastic vasculitis (n=16), pitriazis lichenoides (n=15), drug reaction (n=9), Behçet's disease (n=8), figurate erythema (n=8), panniculitis (n=8), lichen planus (n=7), erythema multiforme (n=6), pigmented purpuric dermatitis (n=5), PUPPP (n=4), Gianotti-Crosti syndrome (n=4), FMF (n=3), spongiotic dermatitis (n=3), arthropod bite (n=2) and 4 other dermatoses.

Conclusions: Lymphocytic vasculitis is believed by some to be the late manifestation of LCV or a non-specific feature but some dermatoses without the characteristic defining pathologic criteria can be diagnosed by this finding. Finding lymphocytic vasculitis in CVD can be a hint for the endothelial cells to be a target, too.

Introduction

Vasculitis is a term defined as the inflammation of the vessel wall which shows some additional features, depending on the diameter of the vessel involved and the type of cells infiltrating the

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vessel. It can be classified by these different perspectives as small, medium-sized, large vessel vasculitis or acute and chronic vasculitis. Leukocytoclastic vasculitis (LCV) is the most commonly seen type of acute, small vessel vasculitis and bears polymorphonuclear leukocytes, nuclear dust, fibrinoid necrosis and destruction of the vessel wall usually along with C3 deposition. Chronic lymphocytic vasculitis (LV) is usually arbitrarily defined by different authors as to have lymphocytes attacking a small vessel, endothelial swelling with or without fibrin deposition. The definitions are criticised for the failure to provide objective diagnosis, because acute vasculitis may progress with time to a chronic stage and fibrin is rarely present in these lesions. Vasculopathic reaction pattern is a general term defining pathologic changes in blood vessels like endothelial swelling and inflammation with extravasated erythrocytes. As far as the controversy about the presence or absence of fibrin is concerned, the diagnosis can be given as "Perivascular dermatitis and vasculopathic changes". This needs dermatologists to be informed about the term and besides, some clinicians would prefer to get an exact diagnosis: "Is it vasculitis or not?". It may be reasonable not to use rigid criteria for the diagnosis of LV since otherwise 'perivascular dermatitis' becomes an underestimation of changes.

There are clinically heterogeneous group of diseases which may present as LV which include pigmented purpuric dermatoses, connective tissue diseases and drup eruptions among a long list, members of which can arbitrarily change depending on the author or the center in concern [1].

Three forms of lymphocytic vasculitis are defined as angiodestructive form, lichenoid lymphocytic vasculitis and lymphocytic endovasculitis. Angiodestructive form is usually seen in lymphoproliferative disorders. Lichenoid form is seen in inflammatory skin diseases as part of the pathologic features which are often characterized by lichenoid vacuolar change and erythrocyte extravasation. Endovasculitis aheads of thrombosis in obliterative conditions [2].

Materials and Methods

In this study, we examined our 127 cases diagnosed as LV retrospectively trying to classify them according to the clinical setting. The histopathological diagnosis was given to the lesions with angiotropism/diapedesis by lymphocytes, erythrocyte extravasation and swelling of endothelial cells, with/without fibrinoid necrosis of the vessel wall.

Results

All cases were sent to our pathology laboratory with clinical diagnoses of diseases which are commonly encountered to present as LV, like the generic term collagen vascular disease including dermatomyositis, lupus erythematosus and morphea; or some rarely applicable causes of LV like arthropod bite and spongiotic dermatitis (Figure 1).

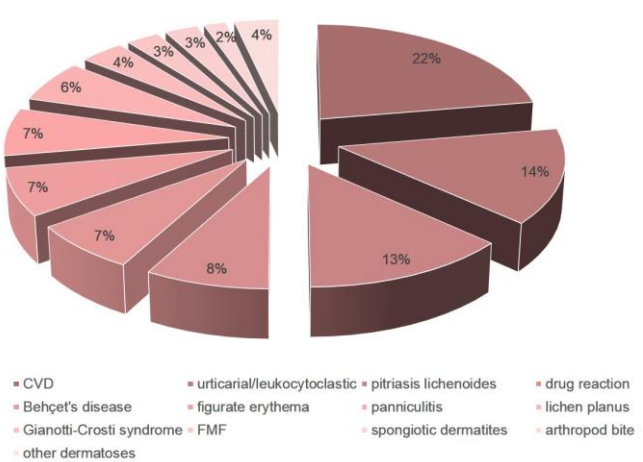

Figure 1. The distribution frequencies of clinical entities with a pathologic diagnosis of LV.

The following are four cases with different underlying causes but the same pathologic presentation as LV.

Case 1. A 31-year-old male patient had had pain on his heel twice a year for the past 4 years, and it had become permanent in the last two months. He had oral aphtous ulcers for the last 6-7 years. He had no genital ulcers or arthralgia. On blood examinations, C-reactive protein was 8.56 mg/dL (normal 0-0.8), hepatitis and HIV serology, as well as autoimmune antibody markers (such as anti-ds-DNA, ANA, ENA) were all negative. Upon administration, he had a subcutaneous nodule on the skin overlying his left gastrocnemius muscle. Clinical diagnosis was Behçet's disease. Biopsy revealed LV of small caliber vessels in the subcutaneous tissue septa without the whole picture of erythema nodosum like panniculitis which can be seen in Behçet's disease (Figure 2). Patient was followed up as such.

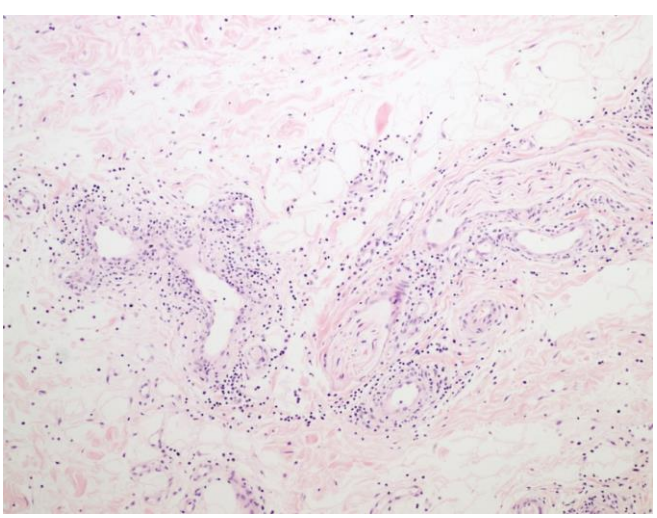

Figure 2. Lymphocytes at the periphery and the wall of the vessels in the subcutaneous tissue septum. Fibrin deposition or classical erythema nodosum picture are not seen (H+Ex100).

Case 2. A 29-year-old woman gave birth one month ago. At the end of postpartum first month, she had increasing pruritus and rash for 5 days. On dermatological examination, there were bilateral erythematous papules and plaques on her trunk. Clinical diagnoses were pruritic urticarial papules and plaques of pregnancy (PUPPP), pemphigoid (herpes) gestationis. Biopsy revealed LV, few eosinophil leukocytes and intraepidermal collection of Langerhans cells consistent with PUPPP (Figure 3). It was her first pregnancy, the rash resolved spontaneously and she did not have a similar eruption in her second pregnancy.

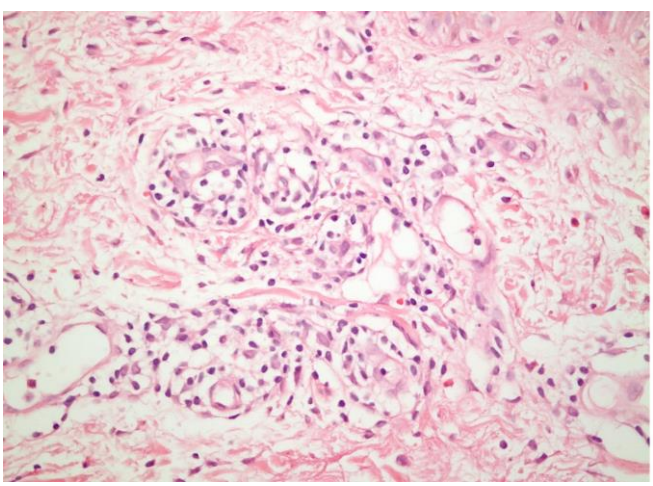

Figure 3. Vacuolization of the endothelia, erythrocyte extravasation and few eosinophils. (H+Ex400).

Case 3. A 23-year-old woman had erythematous papules predominantly on bilateral upper and lower extremities, declining steadily on her trunk. She had no vesicule or pustule formation. Oral mucosa was normal. On her blood test; autoimmune serology (such as Anti-cardiolipin IgM and IgG; Anti-Phospholipid IgM and IgG, Anti-ds-DNA), hepatitis and HIV serology were negative. EBV EBNA IgG was 682 RU/mL and EBV VCA IgG was 2657 RU/mL. Biopsy was taken from her forearm with the clinical diagnoses of viral eruption and Gianotti-Crosti syndrome. Pathologic examination revealed lichenoid vacuolar changes at the interface along with spongiosis and LV consistent with Gianotti-Crosti syndrome (Figure 4)

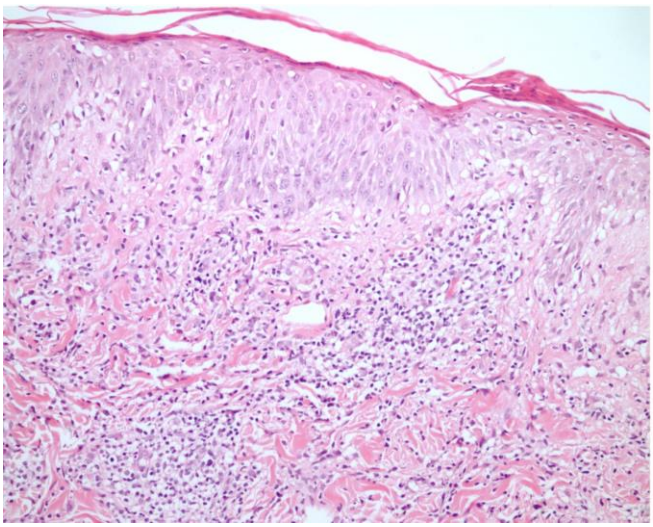

Figure 4. Basal vacuolization of the epidermis, dense lymphocytic reaction around and on the wall of the vessels in the papillary dermis. (H+Ex200).

Case 4. A 39-year-old male patient with a diagnosis of acute myeloid leukemia (AML) had palpable purpura on his bilateral lower extremity after the first dose of cytarabine therapy. Clinical picture was that of LCV, which is usually seen in sepsis or due to medications in these patients. Biopsy findings showed LV (Figure 5)

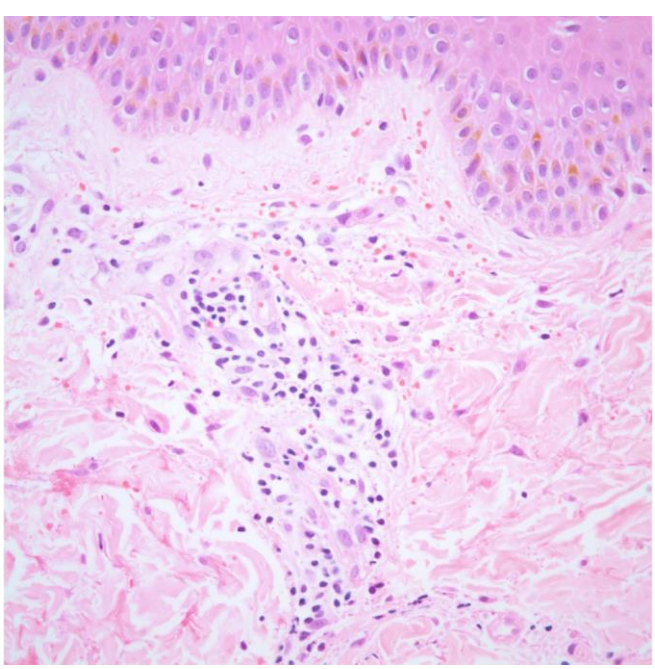

Figure 5. Endothelial swelling, lymphocytic infiltration of the vessel wall and extravasated erythrocytes. Leukemic infiltration is not present (H+Ex200).

Discussion

Vasculitis is a generic term for the inflammation of vessel walls. Many pathologists hesitate to give this diagnosis without a definable clinical condition explaining the presence of vascular damage. Also they should give explanatory notes about the type of vessel and the cellular constituents. A diagnosis of LCV is simpler for the clinician who is familiar with the underlying conditions than the diagnosis of LV which creates a confusion for how to find an appropriate place for this diagnosis in the clinical context for that particular patient. Since LV can have different clinical presentations, it is expected to have many different inflammatory skin diseases or vasculitic conditions included in the differential diagnosis. A pathologist almost never receives a clinical diagnosis of LV in the biopsy form but gives it as a diagnosis. For the pathologist's point of view, LV is a descriptive term defining a morphological change; the etiology leading to vascular damage, which is inflammation. This can fit to many situations [3].

If the term LV vasculitis is used by strict criteria, namely the presence of fibrin, then one should use the term vasculopathic reaction pattern for the lymphocytic reaction together with endothelial swelling or thickening of the vascular wall. This will lead to rarity of this diagnosis and perivascular dermatites will be assumed more important than they mostly are. Diseases showing vasculopathic reaction pattern can be listed as non-inflammatory purpuras, vascular occlusive diseases, urticarias, neutrophilic dermatoses and vasculitis (acute, chronic lymphocytic, granulomatous). The most important category

within this tissue reaction pattern is vasculitis. Then time comes to question the criterion for the vasculitis [4,5].

Chronic LV is a term used for a number of clinically heterogenous diseases. It is characterized by predominantly lymphocytic infiltrate involving and surrounding the small vessels in the dermis. There can be acute or chronic damage to the small vessel walls with fibrin deposition and/or lamination by pericytes. It is usually associated with endothelial cell swelling and erythrocyte extravasation. Nuclear dusting is uncommon. Acute vasculitis may progress with time to a chronic stage and fibrin is rarely present in these late lesions.

Regarding our cases, the distribution frequency of clinical conditions diagnosed pathologically as LV was within the expected range. Collagen vascular disease is a known and top list condition associated with LV [1]. Twenty-two percent of our cases had this diagnosis. One patient in this group had also myelodysplastic syndrome and one had colon carcinoma.

The percentage of the second most common clinical diagnosis, urticarial / LCV (14%) seems to be higher as compared to previous studies. The LV in these cases could represent the late manifestation of LCV [6]. Three patients in this group had accompanying lymphoma, AML and chronic renal failure.

Pitiriasis lichenoides, which makes up 13% of our cases is the prototype of lichenoid LV pattern. One patient had previously diagnosed as mycosis fungoides, 2 had colitis. Graft versus host disease (GVHD) which is said to be the first defined condition with a lichenoid LV was not present in our series. Patients who were diagnosed as GVHD in our department were usually in early phases with grade 2 features and probably the diagnostic / differential diagnostic work-up of GVHD did not involve searching for LV.

Drugs such as aspirin, paracetamol, lipid-lowering agents or herbal medicine may lead to lesions caused by LV [7]. Nine cases (8%) in our series had the clinical diagnosis of drug eruption. One patient was lost to hemophagocytic syndrome.

Behçet's disease, figurate erythema (erythema annulare centrifigum and granuloma annulare) and panniculitis (usually erythema nodosum) made 7% each of our cases.

Lichen planus and spongiotic (nummular) dermatitis were surprisingly present in the clinical diagnoses. These cases may suggest the dominance of vascular changes albeit the minor changes in the epidermis and the interface.

LCV bears neutrophils in the infiltrate and denotes an acute reaction. LV on the other hand, has lymphocytes which are cells capable of recruiting other inflammatory cells (neutrophils and histiocytes). Lymphocytes themselves are masqueraded in conditions like (late phase) LCV and granulomatous vasculitis. LCV is an immune complex mediated reaction and sometimes it can be seen in non-immunological conditions like with bacterial toxins and erythema elevatum diutinum. LV can represent the resolving phase of neutrophilic vasculitis after 24-72 hours. It is a cell mediated reaction causing its effects by cytotoxicity [2].

Conclusions

LV is an important part of diagnostic practice in dermatopathology since it can present as an heterogeneous group of diseases some of which do not manifest themselves clearly. The pathogenesis of LV interests researches since it has been shown that some molecular markers differ in LCV and LV.

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