Human Pathology (2009) **40**, 1224–1233

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Expression and prognostic significance of metalloproteases and their inhibitors in luminal A and basal-like phenotypes of breast carcinoma☆

Luis O. González MD[a,b,c], María D. Corte PhD[a,b], Sara Junquera MD[a], Raquel González-Fernández PhD[d], José M. del Casar PhD[a,b,e], Carmen García MD[a], Alejandro Andicoechea PhD[a,b,e], Julio Vázquez PhD[a,b], Román Pérez-Fernández PhD[d], Francisco J. Vizoso PhD[a,b,e,*]

[a]*Unidad de Investigación, Hospital de Jove, 33290 Gijón, Spain*
[b]*Instituto Universitario de Oncología del Principado de Asturias, 33006 Oviedo, Spain*
[c]*Servicio de Anatomía Patológica, Hospital de Jove, 33290 Gijón, Spain*
[d]*Departamento de Fisiología, Facultad de Medicina, 15782 Universidad de Santiago de Compostela, Spain*
[e]*Servicio de Cirugía General, Hospital de Jove, 33290 Gijón, Spain*

Received 2 September 2008; revised 1 December 2008; accepted 3 December 2008

Keywords:
Breast cancer;
Matrix metalloproteases;
Metastasis;
Tissue inhibitors of matrix metalloproteases

Summary To analyze the expression and prognostic value of matrix metalloproteases and their tissue inhibitors in luminal A and basal-like breast carcinomas, an immunohistochemical study was performed on cancer specimens from 93 randomly selected patients with invasive primary ductal tumors of the breast (46 with and 47 without distant metastasis) and with luminal A (n = 48) (ER+, HER2−) or basal-like (HER2−, ER−, PgR−) (n = 45) lesions. Luminal B cases were too few to analyze. Specimens were also studied using tissue microarrays and specific antibodies against matrix metalloproteases 1, 2, 7, 9, 11, 13, and 14 and tissue inhibitors 1, 2, and 3. There were no significant differences in matrix metalloprotease or tissue inhibitor expression in the 2 phenotypes of tumors. In basal-like carcinomas, high scores for matrix metalloproteases 9 and 11 were significantly associated with a high distant metastasis rate. Likewise, data showed associations between matrix metalloprotease/tissue inhibitor expression by either stromal fibroblasts or mononuclear inflammatory cells and distant relapse-free survival in both tumor phenotypes. In addition, in infiltrating luminal A and basal-like tumors, we identified a prometastatic phenotype of mononuclear inflammatory cells, showing a high matrix metalloprotease/tissue inhibitor molecular profile. Expression of matrix metalloproteases and tissue inhibitors is related to the characteristics of breast tumor cells. As prognostic factors in breast carcinomas of both luminal A and basal-like phenotypes, our results point to the importance of the expression of matrix metalloproteases and tissue inhibitors by the stromal cells.

☆ This work was supported by a grant from FIS-PI040137 Fondo de invesrsión Sanitaria del Instituto Carlos III (FIS-Spain) and Obra Social Cajastur.
* Corresponding author. Hospital de Jove, Servicio de Cirugía General, 33290 Gijón, Spain.

0046-8177/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.humpath.2008.12.022

1. Introduction

Breast cancer is the quintessential sickness affecting women in industrialized countries (22% of all cancers). The mortality rate has been stable for several years, in spite of the increasing incidence of the disease. Nowadays, breast cancer is the second leading cause of cancer death. Clinicians are faced with the challenge of accurate diagnosis. Although clinical signs of disseminated disease occur in fewer than 10% of women at the time of diagnosis, in about half of apparently localized tumors, the disease relapses in the form of metastases within 5 years after surgery.

It is difficult to predict the occurrence of distant metastases because breast cancer is a heterogeneous disease encompassing complex pathologic entities. Even in patients with apparently similar clinical profiles, there is a range of clinical behaviors that influence the accuracy of prediction of distant metastases. This is why data such as nodal status, tumor size, grade, patient age, and tumor hormone receptor status as prognostic variables seem insufficient. New prognostic factors are indispensable to improve the classic risk classification.

Recent DNA microarray profiling of frozen breast cancer samples has identified distinct subtypes associated with an array of clinical outcomes [1-3]. Using an intrinsic set of 534 genes, Sorlie et al [2] analyzed the expression profiles of 115 independent tumor samples, categorizing them into 4 groups: luminal (estrogen receptor positive [ER+]), HER2 over-expressing, normal breast like, and basal like. Basal like is triple-negative breast cancer characterized by absence of ER, progesterone receptors (PgR), and HER2 [2]. The molecular subtype and prognostic expression profile of a primary breast tumor were shown to be maintained throughout its metastatic process [4]. Several other studies have shown the potential clinical value of this classification. For instance, the basal-like subtype comprises about 19% of the tumors. They have a poor prognosis, as assessed by relapse-free survival [2,5,6]. Additional opportunities to identify or validate molecular signatures are provided by molecular profiling using a limited number of protein biomarkers. Thus, basal epithelial cells can be stained with antibodies to keratins 5/6, whereas luminal epithelial cells can be stained with antibodies against keratins 8/18 [7-9]. The prevalence of basal-like breast cancers and their poor prognosis have been validated by recent immunohistochemical studies on formalin-fixed paraffin-embedded archival tumors [7,8,10,11]. The roles of molecules in tumor, the metastasis development, and the phenotypes of breast carcinomas are yet to come to the forefront of cancer research; we also need to expand matrix metalloprotease (MMP) research.

The human MMP family currently consists of 28 members of homologous zinc-dependent endopeptidases. These can either be divided into 8 structural classes or grouped depending on their substrate specificity and primary structure. Finally, MMPs can be clustered into subgroups of collagenases (MMP-1, 8, and 13), gelatinases (MMP-2 and 9), stromelysins (MMP-3, 10, 11), membrane-associated MMPs (MMP-14, 15, 16, 17, 23, 24, and 25), and other novel MMPs [12,13].

MMPs are synthesized as inactive zymogens, which are activated by other MMPs or by serine proteases in a predominant and pericellular manner. Their activity is specifically inhibited by metalloprotease tissue inhibitors (TIMPs) or by nonspecific protease inhibitors (eg, α_2-macroglobulin). Inhibitors 1, 2, 3, and 4 are the 4 TIMPs known to exist. The balance between MMPs and their inhibitors is essentially altered in those physiologic conditions where rapid remodeling of extracellular matrix happens. Cancer is a result of pathologic cellular growth. Interestingly, MMPs are expressed by different tissues at various stages of their development and are conspicuously absent in normal cells of adult organisms [14]. Growth factors and cytokines secreted by either tumor or stromal cells [15] regulate the expression of MMPs in neoplastic tissues in a paracrine manner. That MMPs promote metastases exclusively by modulating the remodeling of extracellular matrix is a dogma easily challenged by the robust data available. Researchers also have been able to identify MMPs' ability to impact tumor cell behavior in vivo through their ability to cleave growth factors, cell surface receptors, cell adhesion molecules, and chemokines/cytokines [16-18]. Furthermore, MMPs may produce a more aggressive phenotype via generation of apoptosis-resistant cells by cleaving proapoptotic factors [19]. MMPs may also regulate angiogenesis in cancer in 2 contrary directions: positively, through their ability to mobilize proangiogenic factors [20], and negatively, by generating angiogenesis inhibitors, such as angiostatin and endostatin, which are then cleaved from large protein precursors [18]. Consequently, several MMPs, in particular, the gelatinases MMP-2 [19-24] and MMP-9 [23,25], have been studied as prognostic factors in breast cancer and are associated with a poor outcome in various subsets of patients. Likewise, several other MMPs, such as MMP-7 [26], MMP-11 [24,26], MT1-MMP (MMP-14) [25], and MMP-13 [27], may be overexpressed or related to clinical outcome in breast cancer. On the other hand, it is now assumed that TIMPs are multifactorial proteins involved in the induction of proliferation as well as in the inhibition of apoptosis [28,29]. Thus, some TIMPs, such as TIMP-1 [30,31] or TIMP-2 [25,32], may be overexpressed or related to clinical outcomes in breast cancer.

These researchers feel these findings are relevant to the attempt to characterize cancer cell behavior. One aim of our present study was to analyze the possible relations between the expression of MMPs and TIMPs of clinical interest in breast cancer and the 2 most frequent cancer subtypes according to the new molecular taxonomy: luminal A and basal-like phenotypes. Considering the heterogeneous nature of these tumor subtypes, we also investigated the prognostic value of MMP/TIMP expression in reference to the occurrence of distant metastasis in both subgroups of tumors.