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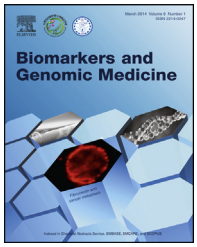

REVIEW ARTICLE

Impacts of protease inhibitors on clathrin and fibronectin in cancer metastasis

Chih-I Wu [a], Ming-Min Chang [a], Chun-Li Su [b], Pin Ling [c], Wen-Tsan Chang [a], Hung-Chi Cheng [a,*]

[a] *Department of Biochemistry and Molecular Biology, College of Medicine, National Cheng Kung University, Tainan, Taiwan*
[b] *Department of Human Development and Family Studies, National Taiwan Normal University, Taipei, Taiwan*
[c] *Departments of Microbiology and Immunology, College of Medicine, National Cheng Kung University, Tainan, Taiwan*

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Abstract Metastasis is a major cause of cancer deaths. Seeking alternative prognostic biomarkers may enable oncologists to make accurate therapeutic decisions to benefit cancer patients. Cumulated evidence reveals that fibronectin (FN) is highly correlated with cancer metastasis but has not been deemed as a prognostic biomarker due to its broad tissue distribution patterns and complicated physiological and pathological functionalities that significantly interfere with the judgmental accuracy. Combining other FN-related factors may make FN possible as a useful prognostic biomarker. Clathrin, a highly protease-susceptible cytoplasmic molecule, is known to affect pericellular FN (periFN) assembly via regulating cell surface FN receptors or FN matrix turnover by coating the endocytic vesicles. Researching our previously published proteomics database of 660 differential secretome proteins expressed in human lung adenocarcinoma cell lines and performing double immunofluorescent staining for periFN and clathrin, we recognized an inverse relationship between them. However, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) data contradicted this relationship, which could be corrected by the addition of a mixture of protease inhibitors into nonmetastatic cancer cell lysates. These results suggested that nonmetastatic cells express either higher levels of cellular proteases or less amounts of protease inhibitors. By examining our proteomic database and reviewing the literature, we conclude that clathrin expression and assembly is inversely correlated with metastatic potential of FN^{high} cancer cells mainly related to the expression of protease inhibitors, instead of proteases. It is worth investigating whether

* Corresponding author. Department of Biochemistry and Molecular Biology, College of Medicine, National Cheng Kung University, 1 University Road, Tainan 70101, Taiwan.
E-mail address: hungchi@mail.nkcu.edu.tw (H.-C. Cheng).

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such an inverse relationship between FN/protease inhibitors and clathrin in human cancers could clinically be incorporated into the prognostic strategy for various cancer types.

Introduction

Metastasis is the major cause of cancer death.[1,2] Prediction of the cancer malignancy at cancer patients' early-onset time may benefit patients by enabling a suitable cancer therapeutic strategy.[3] Unfortunately, clinical and pathological staging proved to be less beneficial.[3] Seeking alternative prognostic biomarkers has thus become urgent to help oncologists make accurate and appropriate therapeutic decisions and to prevent increasing cancer mortality.[4] Recently, accumulating cancer research findings have indicated that fibronectin (FN) expression in various tumor cells is highly correlated with malignant phenotypes and poor prognosis.[5–9] Therefore, it seems that FN could serve as an ideal prognostic biomarker for predicting the tumor progressive outcomes.[5,9] We have previously examined the differential protein expressions in a pair of human lung adenocarcinoma cell lines using the proteomics analysis methodology and identified pericellular FN (periFN) assembly as an important pro-metastatic cellular activity.[10] However, due to the broad tissue distribution patterns and complicated physiological and pathological functions of FN,[6] it is difficult to make a clear diagnostic judgment solely by the FN expression profile on clinical tumor samples as to the effects of FN on tumor progression.[2] Nevertheless, the ultimate goal of using FN as a prognostic biomarker may still be made possible through combining other FN-related factors. Clathrin-dependent endocytosis is essential for the periFN assembly on tumor cell surfaces, either by regulating the activities of cell surface FN receptors, e.g., integrins, or by influencing periFN turnover.[11,12] Importantly, clathrin proteins are known to be highly susceptible to proteolytic cleavages.[13] Here, we further looked into our previous proteomic database[10] and recognized the upregulation of protease inhibitors in highly metastatic and clathrin heavy chain in non-metastatic lung cancer cell lines.[10] This article systematically reviews and integrates the scientific consensus from literature to solve the seemingly contradicted results regarding the relationship between clathrin expression and periFN-promoted cancer metastasis and, based on our results, to rationalize how expression of cellular protease inhibitors, rather than proteases, could contribute to the metastatic phenotypes of cancer cells for future prognostic purposes. Altogether, we propose that high levels of periFN expression/assembly and protease inhibitors may be combined with decreased clathrin expression to serve as concerted malignancy-prognostic biomarkers, which warrants further scientific investigations and scrutiny.

FN and cancer metastasis

FN plays an important role in mediating tumor progression due to its high abundance within extracellular matrices, in the circulation, and on tumor cell surfaces, and its interaction with various cellular components.[14–16] Cumulated evidence manifests the promoting role of FN in cancer metastasis.[1–3,5–9,17–19] We have shown that suspended cancer periFN matrix assembly is required for cancer metastasis via adhering to endothelial dipeptidyl peptidase IV (DPP IV).[6,8,17] The specificity of DPP IV/periFN adhesion in cancer metastasis was demonstrated with the reduced metastatic ability of the tumor cells pretreated with soluble DPP IV prior to intravenous injection,[6,20] or with that injected into Fischer 344/CRJ rats, a Fischer 344 rat substrain with a significantly decreased lung endothelial DPP IV expression.[21] We further employed proteomics analytical tools to investigate how periFN assembly is regulated and identified a trypsin inhibitor, α1 antitrypsin (A1AT; serpine A1) within secretomes of lung adenocarcinoma cells as a required extracellular regulatory protein.[10] Others have also continued to use genomic as well as proteomics approaches and provide evidence that FN expression in cancer cells is highly correlated with cancer metastatic potential and poor prognosis.[2,4,9,18,22–24] For example, by genomically analyzing highly metastatic melanoma cancer cells that were selected with an *in vivo* scheme, FN was identified on the top of a list of gene expressions that correlates with progression to a metastatic phenotype.[2] In the SATB1-reprogramming repertoire that leads to breast tumor growth and metastasis, FN was genomically identified as a highly upregulated gene.[22] For renal cell carcinoma patients, elevated plasma levels of cellular FN, which normally is not present in the plasma, is acceptable and useful as a follow-up prognostic tool to predict more advanced diseases.[18,19] Using proteomics analyses on microvesicular proteins secreted by human breast cancer cells and glioma cells and body fluid, i.e., urine, from human bladder cancer patients, FN has been highly correlated with tumor metastatic ability and poor prognosis.[23,24]

Regulations of periFN assembly and endocytosis

FN matrix assembly is an outcome of steady dynamics and could be augmented by binding of soluble FN to cell surface FN receptors, e.g., integrins $\alpha5\beta1$, $\alpha IIB\beta3$, $\alpha v\beta3$, or syndecans, via inside-out signaling regulation of FN receptor activity.[25–27] Conversely, the established periFN matrices