Irit Sagi

Silvina Federman
Lisa Miller

Studies of ECM invasion by cell-surface activated matrix metalloproteinases using infrared micro-spectroscopy

Department of Structural Biology

Tel. 972 8 934 2130 **Fax.** 972 8 934 4154
E-mail: irit.sagi@weizmann.ac.il

The extracellular matrix (ECM) is a dynamic, macromolecular three-dimensional structure that controls the interactions among cells and the organization of tissues. It is composed mainly of collagen and proteoglycans molecules, which together form a mesh environment of fibers and gel-like structures. Remodeling and proteolytic cleavage of ECM components by MMPs modify cell-ECM interactions and are related to normal physiological processes, such as embryonic development, wound healing, bone growth, and pathological conditions, e.g., those processes related to chronic inflammation and tumor migration. Matrix metalloproteinases (MMPs) constitute a large family of secreted soluble and cell-surface zinc-dependent endopeptidases that cleave ECM components. MMPs are structurally related and can be classified according to their primary structure and substrate specificity: collagenases (MMP-1,8,13), gelatinases (MMP-2,9), stromelysins (MMP-3,7,10,11,12), and membrane type (MT)-MMPs (MT1,2,3,4,5,6-MMP). They are secreted as latent pro-enzymes that are proteolytically activated in the intracellular or extracellular space. Once activated, they are specifically inhibited by tissue inhibitors of metalloproteinases (TIMPs). Recent studies have shown that MT1-MMP on the cell surface mediates the activation of pro-MMP via TIMP-2 mediation. These studies imply that ECM degradation, for the purpose of facilitating cell invasion or migration, should be confined to the immediate pericellular environment of cells. Specifically, it was suggested that MMP-2 activity at the cell surface, and not the soluble enzyme, is critical for remodeling of the ECM by tumor cells. However, our current basic understanding of the mode of action of "cell-surface" activated MMPs on the intact ECM is not sufficient. Two questions to be answered are: (1) Do proteases possess a long-distance ECM remodeling activity? and (2) How do MMPs degrade the complex ECM network?

The focus of our studies is the elucidation of the chemical and structural modifications of intact models of ECM upon degradation induced by cell-surface-activated MMPs. To provide new insights to these questions, we have developed procedures to apply Fourier Transform Infrared (FTIR) micro-spectroscopy as a novel structural-spectroscopic approach that allows us to closely track the remodeling effect of the pericellular proteolysis process on intact ECM matrices and to extract both structural and chemical quantitative information. FTIR spectroscopy is an excellent technique for examining structural changes in proteins, due to the sensitivity of the protein's spectral characteristics to its secondary structure. Combining with a microscope it allows us to measure FTIR spectra with spatial resolution. Thus, FTIR micro-spectroscopy permits direct tracking of the degradation products along the enzymatic catalysis pathways on the biological matrix, and provides us with the means to detect distinct patterns of enzyme degradation on ECM matrices. Our experimental system consisted of matrigel as the biological matrix and HT-1080 cells. Matrigel is a basement-membrane matrix and is usually used as a physiological model system of ECM invasion. HT-1080 are human fibrosarcoma cells, which are characterized as highly invasive and as producing high quantities of detectable MMP-2, MT1-MMP, and TIMPs. The cells were plated on matrigel matrices and incubated both in the presence and in the absence of a wide range MMP inhibitor. Figure 1a shows the FTIR spectra of intact matrigel (detected in cell free areas) and degraded matrigel (detected around the cell boundaries). The spectrum of matrigel near the cell boundaries (in black) shows a shift and a peak broadening, which is characteristic of triple-helix unwinding. To track the degradation pathways of matrigel by cell-activated MMPs we first scanned an IR map of matrigel at 7μm resolution, and then generated and IR image, by spectral correlation. The IR image presented in Figure 1b shows the distinct patterns of degraded matrigel as detected around the cell boundaries, where the blue color represents highly degraded matrigel and the red color represents undegraded matrigel. Matrigel proteolysis or remodeling was not detected when the HT-1080 cells were incubated in the presence of inhibitor (figure 1c). This shows that the detected patterns of matrix degradation are directly related to the action of "cell-surface" activated MMPs on the matrigel matrix.

The degree of matrix degradation and the extent of the pericellular enzymatic action on the matrigel matrix can be directly visualized in the IR image. Extensive matrix degradation is observed in specific areas at the cell boundary, which presumably represent the front of the cell, and somewhat less

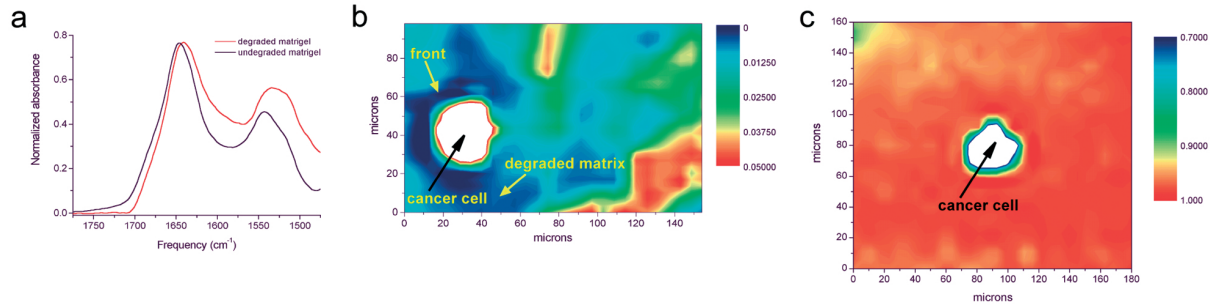

Fig. 1 *(a) FTIR spectra of matrigel in a cell-free area of matrigel (black), and matrigel near the cell boundaries (red). (b) IR image of proteolysis of intact matrigel matrices by cell-secreted MMPs. The blue color indicates degradation and the red color indicates no degradation of the matrix. The cell is encoded in white. (c) IR image of matrigel incubated with HT-1080 cells in the presence of inhibitor shows no matrigel degradation.*

intense proteolysis can be detected around the rest of the cell area. However, a less-intense long-distance proteolysis that is up to 10-20 μm away from the cell boundaries (taking into account the shrinkage of the cells after drying) cannot be ignored and may be involved in destabilization of the ECM network, that enables cell invasion. These results raise the possibility that the mechanisms of MMPs' activation and their subsequent inhibition by TIMP may not be coupled in the initial phase of the invading process. In this case, the first burst of activated enzymes will induce ECM proteolysis in a more diffuse manner and, hence, will not be quenched by local concentrations of TIMPs at the first stages of maturation of active MMP. Consequently, these soluble concentrations of catalytic MMPs will contribute to the general destabilization of the biological matrix within a larger area around the pericellular environment. With time, the major matrix remodeling, achieved presumably by MT-MMPs, will accumulate and will be confined to the cell membrane. Structural characterization of the remodeled matrix macromolecules reveals that the invasion process is achieved mainly by mild proteolysis of the macromolecules within distinct channels in the matrix network, as opposed to evenly spread robust proteolysis of ECM to small polypeptide fragments. Thus, the net enzymatic proteolysis of the matrix may be characterized by destabilization of the local triple helices, as opposed to extensive cleavage of the polypetides within the ECM network.

Acknowledgments

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