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Microscopic Investigations Of DDR1 Oligomerization For Collagen Binding

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Collagen type 1 is the most abundant extracellular matrix (ECM) protein found in vertebrates [1]. The regulation of collagen content is critical for several physiological processes. It is recently being discovered that collagen-binding proteins play an important role in collagen regulation [2, 3]. Discoidin domain receptor 1 (DDR1) is a widely expressed mammalian cell-surface receptors, which binds to and is activated by collagens, including collagen type 1 [4-5]. Little is understood about DDR-collagen interaction and the role of DDR1 oligomerization in its binding to and activation by collagen.

Here we investigated how oligomerization of DDR1 affects its binding to collagen type 1. Utilizing biophysical and microscopy techniques such as surface plasmon resonance (SPR), atomic force microscopy (AFM), transmission electron microscopy and fluorescence light microscopy we have established that oligomerization of DDR1 is critical for its binding to collagen. Our in-vitro assays utilized purified DDR1-Fc proteins [6] consisting of only the extracellular domain (ECD) of DDR1, necessary for collagen binding. DDR1-Fc could be oligomerized by means of anti-Fc antibody. The dissociation constant for DDR1 binding to immobilized collagen 1 was significantly higher when the same amount of DDR1-Fc was in oligomeric form. AFM imaging in a fluid environment could distinguish between DDR1-Fc alone and oligomers of DDR1. DDR1-Fc was found to exist as a dimer and stoichiometry of DDR1-Fc in antibody-mediated oligomers could be determined (figure 1). Further, by AFM imaging we determined that it were DDR1 oligomers which bound to collagen type 1 (Figure 2).

To investigate if DDR1 dimerization is necessary for collagen binding we have created DDR1-His proteins which exist as monomers in contrast to DDR1-Fc. DDR1-His and DDR1-Fc proteins have been used to investigate binding to immobilized and soluble collagen. AFM and TEM have been used to analyze the binding events at the single-molecule level and determine role of DDR1 oligomerization in its binding to collagen. Our investigations reveal that DDR1 oligomerization greatly enhances its binding to immobilized collagen. Further we have evidence that soluble collagen can induce oligomerization of monomeric DDR1 leading to its binding to collagen (figure 2). Our results provide new insights into how DDR1 oligomerization affects its binding to collagen and plays a role in collagen regulation. These results also signify an important role of the naturally shedded ECD of DDR1 which occurs in almost all cell lines [7].

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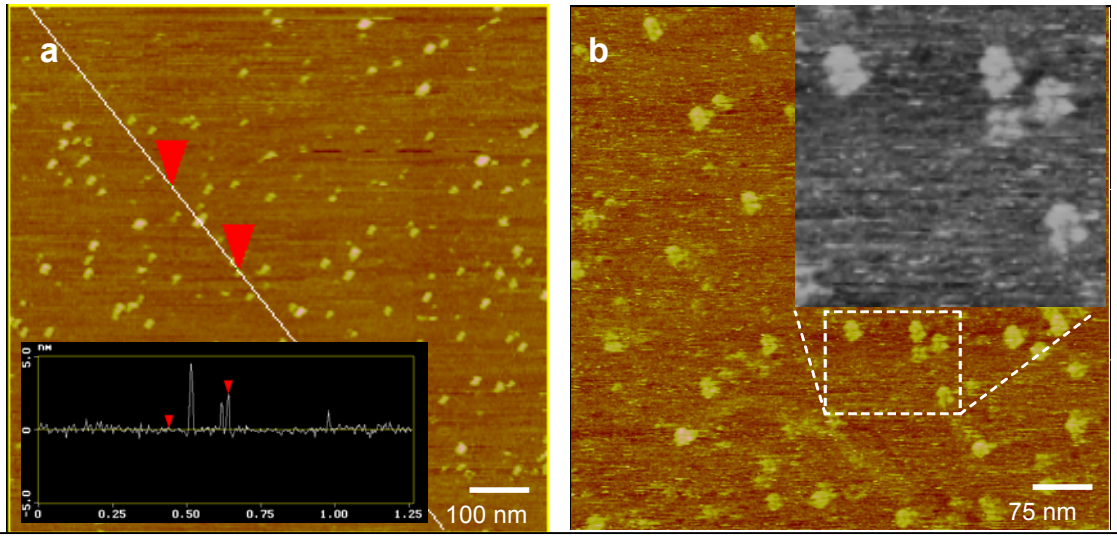

Figure 1: **AFM imaging shows that (a) DDR1-Fc images as a globular protein with two lobes and (b) incubation with anti-Fc antibody leads to oligomerization of DDR-Fc. Oligomers with four globular lobes can be resolved. AFM images were acquired in a fluid environment in tapping mode using a Multimode AFM (Digital Instruments, Santa Barbara, CA).**

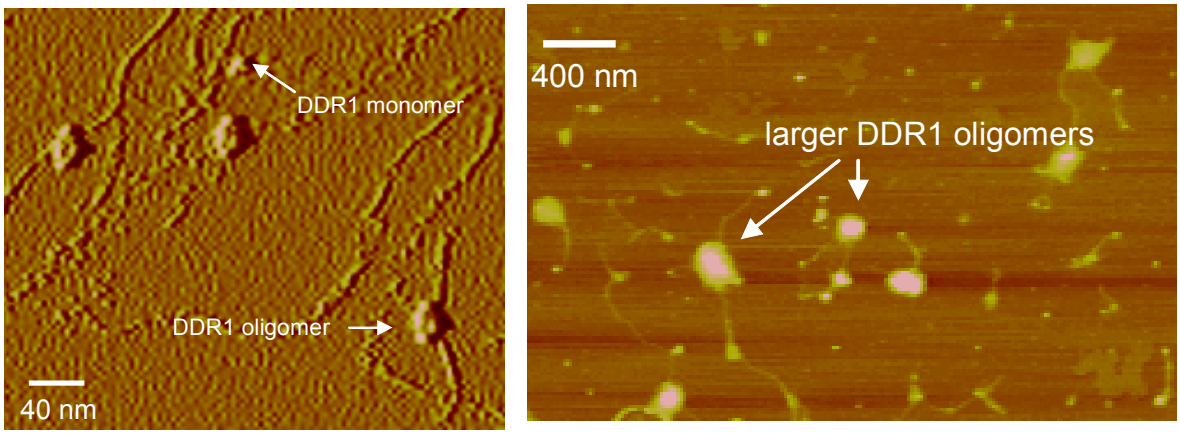

Figure 2: **(Left) AFM image of DDR1 binding to immobilized collagen. DDR1-Fc was oligomerized by means of anti-Fc antibody and incubated over immobilized collagen using methods described earlier [6]. (Right) DDR1 binding to soluble collagen promotes formation of larger oligomers of DDR1**

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