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Inhibition of Collagen Fibrillogenesis by Cells Expressing Soluble Extracellular Domains of DDR1 and DDR2

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Collagen fiber assembly affects many physiological processes and is tightly controlled by collagen-binding proteins. However, to what extent membrane-bound *versus* cell-secreted collagen-binding proteins affect collagen fibrillogenesis is not well understood. In our previous studies, we had demonstrated that the membrane-anchored extracellular domain (ECD) of the collagen receptor discoidin domain receptor 2 (DDR2) inhibits fibrillogenesis of collagen endogenously secreted by the cells. These results led to a novel functional role of the DDR2 ECD. However, since soluble forms of DDR1 and DDR2 containing its ECD are known to naturally exist in the extracellular matrix, in this work we investigated if these soluble DDR ECDs may have a functional role in modulating collagen fibrillogenesis. For this purpose, we created mouse osteoblast cell lines stably secreting DDR1 or DDR2 ECD as soluble proteins. Transmission electron microscopy, fluorescence microscopy, and hydroxyproline assays were used to demonstrate that DDR ECD expression reduced the rate and quantity of collagen deposition and induced significant changes in fiber morphology and matrix mineralization. Collectively, our studies advance our understanding of DDR receptors as powerful regulators of collagen deposition in the ECM and elucidate their multifaceted role in ECM remodeling.

Keywords: DDR1; DDR2; collagen fibrillogenesis; TEM; ECD

Introduction

Collagen fibrillogenesis, the assembly of collagen fibers, is a critical process in the development, maturation, and repair of mammalian tissue. Alterations in the structure and amount of deposited collagen fibers can greatly alter the integrity of the whole tissue. Even a single point mutation in collagen type I can severely compromise the strength of cortical bone tissue leading to osteogenesis imperfecta.[1] Further, the interaction between collagen-binding proteins and collagen molecules during fibrillogenesis can promote significant alterations in the resulting collagen fiber structure and subsequent extracellular matrix (ECM) remodeling.[2,3] For example, soluble collagen-binding proteins such as decorin, biglycan, fibronectin, and vitronectin are thought to play a significant role in the process of collagen fibrillogenesis and bone mineralization due to their interaction with collagen molecules.[4]

The collagen-binding membrane proteins discoidin domain receptors (DDR1 and DDR2) are transmembrane receptors belonging to the family of receptor tyrosine kinases and have been studied for ECM remodeling in atherosclerosis,[5–7] osteoarthritis,[8–10] and several malignancies.[7] It is

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Abbreviations used: DDR, discoidin domain receptor; ECD, extracellular domain; ECM, extracellular matrix; KD, kinase deficient; TEM, transmission electron microscopy; HP, hydroxyproline; FITC, fluorescein isothiocyanate; SMC, smooth muscle cell.

well established that activation of the DDR1 and DDR2 kinase domain up-regulates the expression of various matrix metalloproteinases[5,11] and alters the biosynthesis of collagen.[5] The extracellular domain (ECD) of DDRs is known to be necessary and sufficient for its interaction with collagen.[11] Besides the full-length receptor, the DDR1 ECD is also found in five distinct isoforms[12] and as a shed protein in the ECM.[13,14] Several protein[5] and mRNA[15–17] species consisting of the DDR2 ECD have also been observed *in vivo*. However, the functional roles of these ECDs of DDRs lacking their kinase domain are not well understood. We had previously elucidated that DDR1 ECD[18] and DDR2 ECD[19] inhibit collagen fibrillogenesis *in vitro* when used as purified proteins. Further, we have recently demonstrated that the DDR2 ECD when anchored on the cell surface preserves the capacity to inhibit collagen fibrillogenesis independent of its kinase activity.[2] It is therefore likely that the expression of soluble ECD of DDRs by cells such as those found in the shedding of DDR1 ECD[12] may play an important role in matrix remodeling.

The fibrillogenesis process of collagen is understood to initiate in the extracellular space near the plasma membrane where secretory vesicles form regions of deep invagination.[20] However, it is not clear how and when collagen-binding proteins interact with collagen molecules during fibrillogenesis or to what extent membrane-bound *versus* soluble collagen-binding proteins affect the collagen fibrillogenesis process by cells. In this study, we seek to elucidate the alterations in collagen fibrillogenesis arising due to soluble DDR1 and DDR2 ECDs secreted by the cells and compare the results with our previous findings utilizing the kinase-deficient, membrane-bound DDR2 ECD (DDR2/-KD). Similar to our previous study, we created stably transfected mouse osteoblast cell lines to express DDR1/ECD or DDR2/ECD as a soluble protein. We utilized a number of ultrastructural and biochemical analyses to elucidate how alterations in the collagen matrix, due to DDR ECDs, affects collagen fibrillogenesis and matrix mineralization.

Results

Characterization of stable cell lines

We used mouse pre-osteoblast cell line MC3T3-E1 to ascertain the effects of cell secretion of DDR1/ECD and DDR2/ECD on collagen fibrillogenesis by the cells. Based on previous studies by us[2] and others[21] these cells secrete and form well-defined collagen fibers in the ECM over a period of one to several weeks. These cells were stably

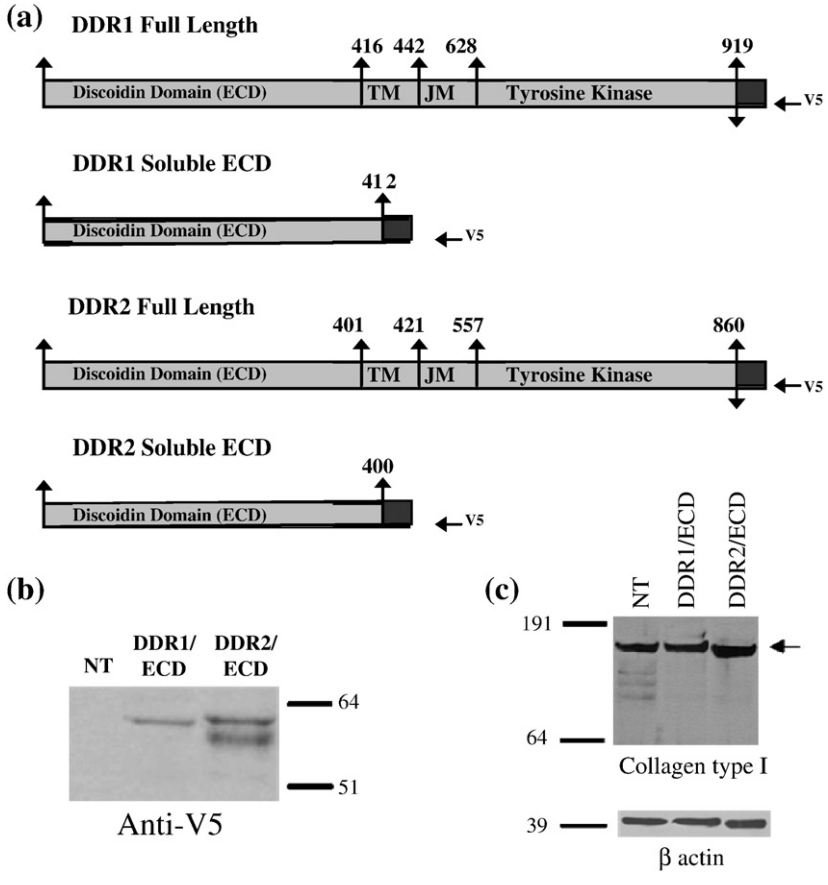

Fig. 1. Creation of stable cell lines expressing the recombinant protein DDR1/ECD and DDR2/ECD. (a) A schematic representation of the V5 His-tagged full-length mouse DDR1 and DDR2 and the V5 His-tagged soluble ECD proteins, DDR1/ECD and DDR2/ECD. (b) Verification of DDR1/ECD and DDR2/ECD in conditioned media of stable cell lines. Western blot analysis using anti-V5 antibodies was performed on conditioned media from each cell line as indicated. Presence of DDR1/ECD and DDR2/ECD was observed at their expected molecular masses, slightly under 64 kDa as a single band (for DDR1/ECD) and as a doublet for DDR2/ECD. (c) Western blotting of whole-cell lysates from nontransfected or stably transfected cells (as indicated) shows no significant difference(s) in collagen expression in the various samples.