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Identification of β1 integrin as mediator of melanoma cell adhesion to lumican

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Abstract

Lumican is a small leucine-rich proteoglycan (SLRP) present in the dermal extracellular matrix. Previous data from our laboratory demonstrated that lumican decreases melanoma progression *in vivo*. Here, we show that melanoma cell migration is decreased by lumican and that this effect is due to an enhanced cell adhesion. The adhesion of A375 human melanoma cells on lumican was dose-dependent and required Mg^{2+} and Mn^{2+} divalent cations. Using a panel of monoclonal antibodies directed against integrin subunits, we showed that A375 cells can bind to recombinant lumican through β1 type integrins. Moreover, the use of rhodocetin, an inhibitor of α2 integrin, suggested that this particular subunit might also be involved in the interaction with lumican. The increased β1 integrin-mediated adhesion of melanoma cells to lumican might explain, at least in part, the anti-invasive effect of this SLRP.

Keywords: Adhesion; Integrin; Lumican; Melanoma; Migration; Rhodocetin; Proteoglycans

Lumican belongs to the small leucine-rich proteoglycan familly (SLRP) [1]. It is present in normal adult human skin as a glycoprotein with a 37 kDa core protein. Generation of knock-out mice has proven the role of lumican in the regulation of the formation of collagen fibrillar networks [2]. Lumican-lacking mice have a fragile dermis due to irregular fibrillogenesis, which might facilitate melanoma progression in the extracellular matrix (ECM). In dermal fibroblasts, *in vitro*, lumican mRNA expression decreases during aging suggesting that the impairment of SLRP synthesis might be involved in the functional alterations of aged skin [3].

In addition to the control of collagen fibril assembly, SRLPs, particularly lumican and decorin, possess antitumor activity. We previously demonstrated that lumican inhibits melanoma progression in a mouse experimental model [4]. In tumor tissues, such as breast cancer [5], lumican mRNA is detected in fibroblasts adjacent to cancer cells. Low expression levels of lumican are associated with poor outcome of invasive breast carcinoma [6].

Many cell–matrix and cell–cell interactions are implicated in the progression and growth of the tumors [7]. The roles of integrins in cell motility, invasiveness, growth, and survival of human melanoma are well established [8–10]. Murine melanoma cells express different integrins, including vitronectin and fibronectin receptors, which are involved in cell migration [11]. The expression of these integrins and their interaction with ECM macromolecules are necessary for proliferation and invasion of solid tumors [12].

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Receptors able to bind lumican on cancer cells are still not known. Here, we showed that human recombinant lumican core protein can inhibit migration of A375 human melanoma cells by increasing their adhesion. Moreover, we provide evidence that A375 human melanoma cells can directly interact with recombinant human lumican core protein *via* β1 integrins subunits.

Materials and methods

Reagents and cells. Recombinant human lumican core protein was produced as previously described [4]. Type I collagen was prepared from rat tendon by extraction with 0.1 M acetic acid [13]. Mouse monoclonal anti-human α1 (FB12), α2 (P1E6), α3 (P1B5), α4 (P1H4), α5 (P1D6), αv (P3G8), αvβ3 (LM609), β1 (6S6), and rat monoclonal anti-human α6 (GoH3) integrins were purchased from Chemicon (Souffelweyersheim,

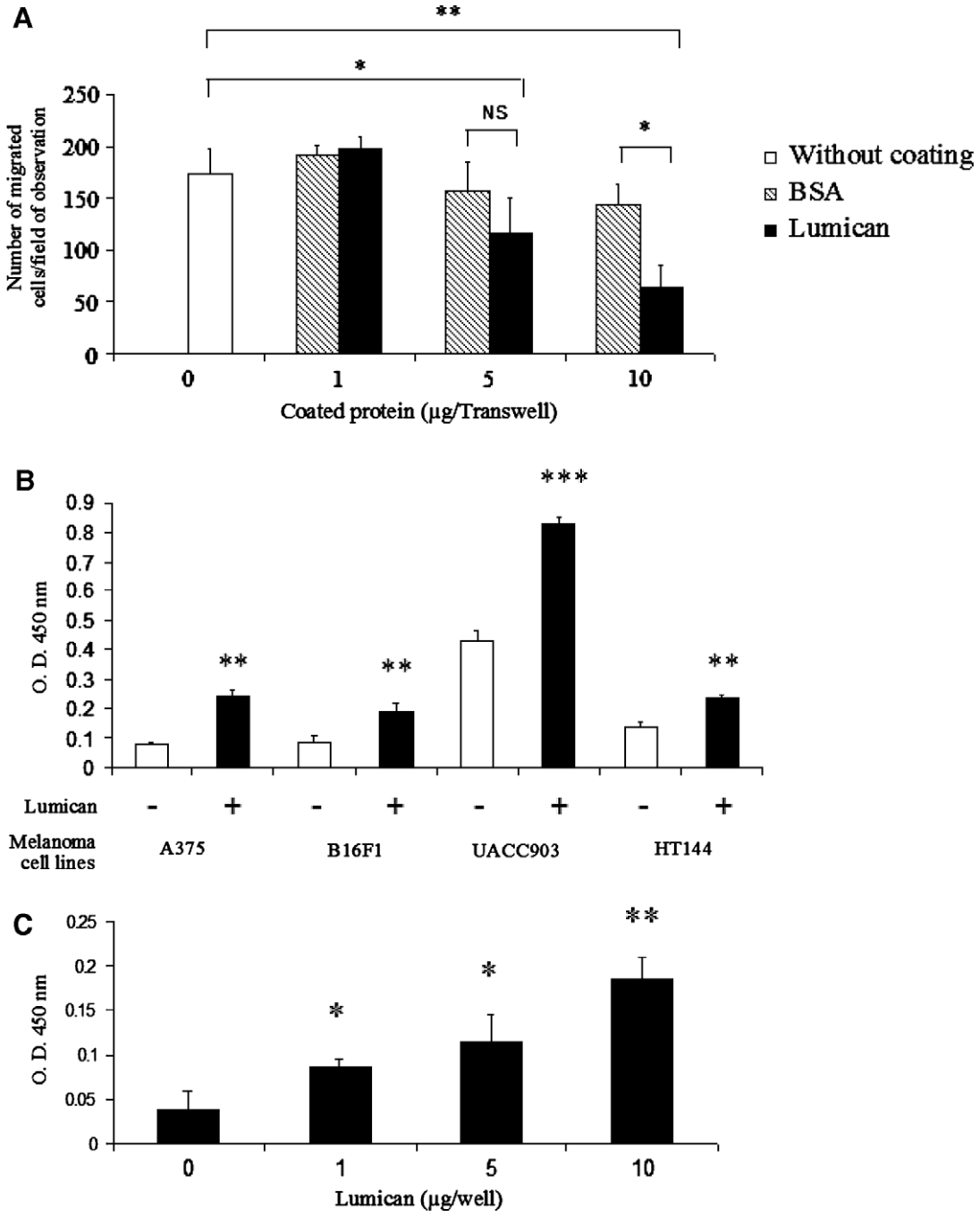

Fig. 1. Recombinant human lumican decreases A375 melanoma cell migration and increases cell adhesion. (A) For migration, 5×10^4 cells were seeded on Transwell® membranes previously coated with 0, 1, 5 or 10 µg of lumican. After 24 h of incubation at 37 °C, cells were stained with crystal violet. The migrated cells were counted in five random fields. (B) Adhesion of different melanoma cell lines to lumican. Cells were seeded in 24-well plates with (black bars) or without (open bars) lumican (60 µg/well). (C) Dose-dependent effect of lumican on adhesion. Cells were seeded in 96-well plates coated with increasing amounts of lumican. They were incubated for 2 h at 37 °C then gently washed with PBS and stained with WST-1 solution as described in Materials and methods. The OD was measured at 450 nm. The data are expressed as the means ± SD from three replicates and are representative of three independent experiments. *Significantly different from the control without lumican ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; NS, not significant).