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Toxicon 50 (2007) 1053–1063

TOXICON

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Effect of RGD-disintegrins on melanoma cell growth and metastasis: Involvement of the actin cytoskeleton, FAK and c-Fos ☆

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Received 8 June 2007; received in revised form 18 July 2007; accepted 19 July 2007
Available online 6 August 2007

Abstract

The effects and molecular mechanisms of RGD-disintegrins isolated from snake venoms on the growth and metastatic potential of B16F10-melanoma cells were investigated. Jarastatin (JT) from *Bothrops jararaca* is a ligand of $\alpha_5\beta_1$, $\alpha_v\beta_3$ and $\alpha_M\beta_2$ integrins, flavoridin (FL) from *Trimeresurus flavoridis* binds preferentially to $\alpha_5\beta_1$ and kistrin (KR) from *Calloselasma rhodostoma* is a selective ligand of $\alpha_v\beta_3$. When injected simultaneously with melanoma cells in mice, the three disintegrins significantly reduced tumor lung colonization. On the other hand, JT and FL, but not KR, inhibited B16F10 cell growth *in vitro*. Interaction of JT or FL with melanoma cells induced actin cytoskeleton rearrangement, increasing actin polymerization and FAK phosphorylation. The effect of FL correlates with the decrease in the constitutively high nuclear content of c-Fos, whereas JT interfered with NF-κB translocation in melanoma cells. None of the disintegrins produced alterations in the nuclear Erk-2. The results provide further evidence to suggest RGD-disintegrins as potent anti-metastatic agents *in vivo*, and indicate that their interaction with $\alpha_5\beta_1$ integrin interfere with integrin-couple signaling, down-regulating transcription factors and negatively modulating cell proliferation. These effects may contribute to inhibition of melanoma cell invasion *in vivo*.

Keywords: Disintegrins; Melanoma cells; Integrin signaling; Metastasis; Proliferation; Nuclear transcription factors; Actin cytokeleton; FAK

1. Introduction

Malignant transformation and development of metastatic processes in melanoma are closely related to alterations in integrin expression as well as integrin-mediated attachment and intracellular

☆ *Ethical statement*: All experiments and procedures were approved by the Institutional Ethics Committee and were conducted in accordance with the National Institute of Health Animal Care Guidelines.

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0041-0101/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2007.07.016

signaling (Kuphal et al., 2005; Varner and Cheresh, 1996; Polsky and Cordon-Cardo, 2003). Mediating cell interactions to the extracellular matrix, the integrins are able to transduce signals by connecting to the cytoskeleton, cytoplasm kinases and transmembrane growth factor receptors (Giancotti and Ruoslahti, 1999; Cary and Guan, 1999; Danen, 2005). Therefore, the integrins play a crucial role in modulating gene expression and regulate cell proliferation, survival and differentiation (Polsky and Cordon-Cardo, 2003; Danen, 2005). The pattern of integrins on cell surface is usually very specific, which makes the cell fit perfectly into surrounding environment. Their expression is frequently changed during malignant transformation, and the up-regulation of integrin expression is associated with metastatic phenotype (Kuphal et al., 2005). For instance, integrins such as $\alpha_V\beta_3$ and $\alpha_5\beta_1$ are present in melanoma cells but do not seem to be expressed in melanocytes, based on *in situ* analysis (Kuphal et al., 2005; Marshall et al., 1998). Binding of specific antibodies or receptor antagonists to $\alpha_V\beta_3$ and $\alpha_5\beta_1$ was reported to inhibit migration, proliferation and metastasis of melanoma as well as of other tumor cells (Trikha et al., 2004; Qian et al., 2005). According to that, the binding of $\alpha_v\beta_3$ integrin to its ligand can induce melanoma cell growth by inhibiting apoptosis (Petitclerc et al., 1999), while the blockade of this integrin on a human melanoma cell line caused inhibition of lung colonization in an experimental metastasis assay (Trikha et al., 2002). On the other hand, the engagement of $\alpha_5\beta_1$ integrin, in the absence of attachment to fibronectin, seems to be related to a decrease on cellular proliferation by blocking transcription of the immediate early genes c-fos, c-jun and jun-B (Varner et al., 1995). From these findings, increasing emphasis has been given on the investigation of novel integrin–ligand molecules as potent therapeutic agents for cancer.

The disintegrins are considered useful tools to better understand the role of integrins in cellular processes. They were originally defined as low-molecular-weight, cysteine-rich proteins containing an RGD/KGD loop, which selectively bind to integrins, being potent inhibitors of platelet aggregation (Niewiarowski et al., 1994). More recently, other tripeptide sequences able to recognize integrins have been identified in some disintegrins (Barja-Fidalgo et al,. 2005; McLane et al., 2004). How integrins modulate melanoma cell responses and signaling has not been fully understood; however, research on disintegrin interactions with melanoma cells has helped to elucidate some obscure issues. Nevertheless, they may also be thought of as models for the design of new therapeutic drugs in the future.

Danen et al.(1998) have demonstrated that eristostatin, an RGD-disintegrin, inhibits melanoma cell metastasis by interfering with $\alpha_4\beta_1$–VCAM binding. Another disintegrin, echistatin, a ligand of $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins, was shown to inhibit tyrosine phosphorylation of focal adhesion components and also induced disassembly of the actin cytoskeleton in fibronectin-adherent melanoma cells (Staiano et al., 1997; Della Morte et al., 2000). Although usually considered as integrin antagonists, interaction of disintegrins with a specific integrin could also trigger intracellular signaling pathways in different cells. Our group has shown in human neutrophils that jarastatin (JT), a ligand of $\alpha_5\beta_1$, $\alpha_V\beta_3$ and also $\alpha_M\beta_2$ integrins, interferes with chemotaxis and apoptosis activating integrin-coupled signaling pathways in human neutrophils (Coelho et al., 1999, 2004, 2001). However, this effect of JT was not shared by other RGD-disintegrins as flavoridin (FL), which binds with higher affinity to $\alpha_5\beta_1$, and kistrin (KR), a selective ligand of $\alpha_V\beta_3$, which activate differently integrin signaling in neutrophils (Coelho et al., 2004). In addition, we have recently demonstrated that FL and KR modulate the activity of the transcription factor AP-1, increasing c-Fos expression and activating human lymphocyte proliferation (Helal-Neto et al., 2007). Therefore, the disintegrins could influence cell function and behavior through the activation of integrin-coupled signaling pathways.

In the present study, we aimed to investigate the effect of three RGD-disintegrins, JT, KR and FL, on melanoma cell growth and metastasis, as well as their effects on integrin-mediated signaling, by analyzing focal adhesion, actin cytoskeleton dynamics and alterations in transcriptional factors on a highly metastatic murine melanoma cell line, B16F10.

2. Materials and methods

2.1. Disintegrins

JT, isolated from *Bothrops jararaca* venom, was purified by reverse-phase FPLC and microsequenced by automated Edman degradation on a Porton integrated microsequencing system, as described earlier (Coelho et al., 1999). KR, isolated