CCN1 is a novel target to reduce the metastasis of melanoma

James Hutchenreuther

Received: 26 September 2013 / Accepted: 27 September 2013 / Published online: 6 November 2013
© The International CCN Society 2013

Abstract Melanoma is becoming increasingly common in recent years and has a very high mortality rate, owing largely to its highly metastatic nature. It tends to metastasize early in the course of the disease, and after metastasis is resistant to most current therapies. Preclinical data has indicated that heparin administered to patients as an antithrombotic treatment also has anti-metastatic properties. Heparin has been shown to interfere with the binding of the integrin Very Late Antigen 4 (VLA-4) to its ligand Vascular Cell Adhesion Molecule 1 (VCAM-1) and in a recent paper the laboratory of Bendas (Thrombosis and Haemostasis, Prepublished online) demonstrated that CCN1 binds to VLA-4 and interference in this binding by heparin results in reduced strength of the VLA-4/VCAM-1 binding. This indicates that CCN1 might represent a good target for reducing the metastasis, and thus mortality, of melanoma.

Keywords Melanoma · CCN1 · VLA-4 · Heparin · Cyr61

In 2013 there will be an estimated 76,690 new cases of melanoma diagnosed in the United States, causing 9,480 deaths. Even though these cases will represent only less than 5 % of all skin cancer diagnoses, they will cause the majority of skin cancer deaths (American Cancer Society 2013). What makes melanoma so lethal is its tendency to metastasize early in the course of the disease and lie dormant in distant parts of the body. In order for a primary lesion to metastasize it must penetrate the dermis, enter the circulatory system, travel and come to a rest in a distant capillary bed, and then begin the process of extravasation (Damsky et al. 2011). The interaction of a high affinity form of the integrin Very Late Antigen 4 (VLA-4) and its ligand, Vascular Cell Adhesion Molecule 1

(VCAM-1), has been implicated in the ability of melanoma cells to adhere to and cross the endothelial layers at sites of metastasis (Klemke et al. 2007).

The activation of integrins like high affinity VLA-4 has been attributed to the activity of matricellular proteins, a class of non-structural protein responsible for bidirectional communication between cells and their surrounding microenvironment. The CCN family of matricellular proteins has been shown to perform many of these functions through direct binding to several distinct integrins and several of the members of the CCN family of proteins, like CCN1 (also known as Cyr61), has been shown to be highly expressed in particularly metastatic forms of melanoma (Ibrahim et al. 2003).

Low Molecular Weight Heparin (LMWH) administered to cancer patients has been shown to increase their survival chances by reducing the incidence of metastasis, and the laboratory of Bendas has previously demonstrated that the anti-metastatic properties of heparin function by preventing the VLA-4/VCAM-1 binding (Fritzsche et al. 2008).

In a recent paper, Schmitz and colleagues test whether binding of heparin to CCN1 plays a role in the interference of LMWH on VLA-4/VCAM-1 by preventing it from activating VLA-4 (Schmitz et al. 2013). This was evaluated by creating a shRNA induced CCN1 knockdown of human MV3 melanoma cells, which are known to use VLA-4/VCAM-1 binding to facilitate their metastasis (Schlesinger et al. 2012). When wild type and CCN1 knockdown MV3 cells were seeded on VCAM-1 the knockdown cells showed reduced adhesion, which was rescued by the addition of recombinant CCN1. The knockdown cells also showed a reduced migration velocity.

In order to characterize the strength and nature of binding between CCN1 and VLA-4 as well as between CCN1 and heparin several mutant varieties of CCN1 were generated, each missing different domains of the protein. CCN1 was found to bind to VLA-4 through a binding site on domain III of the protein, while it binds to heparin through a site on domain IV. Addition of recombinant CCN1 was shown to be able to

J. Hutchenreuther (✉)
Department of Physiology and Pharmacology, Medical Sciences
Building, Western University, London, ON N6A 5C1, Canada
e-mail: jhutche@uwo.ca

increase vinculin staining in both wild type and CCN1 knockdown cells. This effect was prevented by simultaneous addition of CCN1 and LMWH, but not by addition of CCN1 and subsequent addition of LMWH. These results indicate that while heparin does in fact bind CCN1, it is only able to bind free CCN1 and cannot remove those proteins already bound to VLA-4. Finally, a structurally modified version of LMWH which does not directly interact with VLA-4, but still binds to CCN1 was added to wild type and CCN1 knockdown MV3 cells binding to VCAM-1. The modified LMWH reduced the adhesion of wild type MV3 cells without having any effect on the adhesion of the knockdown cells. This suggests that there is a significant difference in adhesion due to the ability of heparin to interfere with CCN1 binding to the cell surface.

To summarize, the paper makes a strong case for CCN1 mediated activity of VLA-4 increasing the metastatic abilities of melanoma by showing a link between CCN1 expression levels and the strength of the binding between VLA-4 and VCAM-1, as well as by establishing a direct binding site between CCN1 and VLA-4. CCN1 is also implicated as a target of heparin binding which results in part of the anti-metastatic actions seen in heparin therapies. While it is beyond the scope of this particular study, the binding of free CCN1 by heparin also indicates there might be an inhibitory effect on the other integrin-mediated tumourigenic effects of CCN1.

CCN1 is a good potential target for the prevention of melanoma metastasis and further research is warranted to

determine if disruption of CCN1 activity translates into reduced metastasis in a biological model.

References

- American Cancer Society. "Melanoma Skin Cancer". Revised May 30, 2013 <http://www.cancer.org/cancer/skincancer-melanoma/detailedguide/index>. Accessed September 20, 2013
- Damsky WE, Rosenbaum LE, Bosenberg M (2011) Decoding Melanoma Metastasis. *Cancers* 3:126–163
- Fritzsche J, Simonas D, Bendas G (2008) Melanoma cell adhesion can be blocked by heparin in vitro: Suggestion of VLA-4 as a novel target for antimetastatic approaches. *Thromb Haemost* 100:1166–1175
- Ibrahim SM et al (2003) Mechanisms of hypoxic gene regulation of angiogenesis factor Cyr61 in melanoma cells. *J Biol Chem* 278: 45651–45660
- Klemke M, Weschenfelder T, Konstandin MH, Samstag Y (2007) High affinity interaction of integrin $\alpha 4 \beta 1$ (VLA-4) and vascular cell adhesion molecule 1 (VCAM-1) enhances migration of human melanoma cells across activated endothelial cell layers. *J Cell Physiol* 212:368–374
- Schlesinger M, Schmitz P, Zeisig R, Naggi A, Torri G, Casu B, Bendas G (2012) The inhibition of the integrin VLA-4 in MV3 melanoma cell binding by non-anticoagulant heparin derivatives. *Thrombosis Res* 129:603–610
- Schmitz P, Gerber U, Schütze N, Jüngel E, Blaheta R, Naggi A, Torri G, Bendas G (2013) Cyr61 is a target for heparin in reducing MV3 melanoma cell adhesion and migration via the integrin VLA-4. *Thromb Haemost* 110